Hepatocytes Transplantation

Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine

Kwang-Woong Lee

Liver transplantation has significantly improved the prognosis in patients with acute liver failure, end-stage liver disease, and metabolic liver disease such as Crigler-Najjar syndrome, urea cycle defects, and familial hypercholesterolaemia.

Acute liver failure, however, is potentially reversible and inborn errors of metabolism often occur in the face of an otherwise normal liver. In addition, the expense and limited supply of donor organs is likely to exclude liver transplantation as an option for most potential patients in the foreseeable future.

Hepatocyte transplantation is a promising new approach for the treatment of liver-based inborn errors of metabolism, or secondary liver function impairment, as is seen acute liver failure or end stage liver disease. Beside curative approach for genetic metabolic deficiencies (familial hypercholesterolemia, hemophilia, etc.), it could be a useful tool for bridging the waiting period until an appropriate donor organ is obtained. Hepatocyte transplantation offers several advantages over whole-liver transplants: it would permit a single donor liver to benefit multiple patients, require a less-invasive surgery, facilitate multiple treatments of a single patient, allow ex vivo genetic manipulation of the hepatocytes before transfer and enable the use of frozen stocks of donor cells.

Since 1969, when a method for isolating primary hepatocytes by collagenase perfusion was developed (1), numerous studies have documented the efficacy of hepatocyte transplantation in the laboratory for the treatment of these disorders. Although animal studies have set the stage for clinical application of hepatocyte transplantation, evidence for long-term survival and function of transplanted human hepatocytes has been tantalizingly slow to come.

In the last few years, a number of investigators have performed hepatocyte transplantation for life-threatening liver failure. Unfortunately, patients with fulminant liver failure have an extremely variable course and can recover spontaneously, so that improvement in survival or successful bridging to whole organ liver transplantation are endpoints that are too ambiguous to convincingly prove the function of transplanted liver cells. In fact, Mito, a leading advocate for hepatocyte transplantation, has concluded that transplanted liver cells are probably not responsible for most of the clinical improvement after
transplantation (2). It was with this background that our research group began their examination of hepatocyte transplantation for the treatment of inherited metabolic diseases, where reconstitution of the defective metabolic function could be determined unequivocally.

Preparation of hepatocytes

The main source of normal human hepatocytes is unused donor livers. Well-established protocols for isolation of hepatocytes are being used (3), they all depend on a collagenase perfusion technique. Once the liver tissue is digested, the hepatocytes are then purified and assessed for cell viability and biological activity. The number of hepatocytes is then estimated and either transplanted into patients or cryopreserved for future use. Hepatocyte cryopreservation techniques are not very well developed yet, however, a sufficient number of viable and biologically active hepatocytes could be recovered from frozen stocks of cryopreserved hepatocytes, which could be used for hepatocyte transplantation (4).

Pre-clinical in vivo studies

A large number of animal models of human liver disease have been studied. These studies have established the feasibility and efficacy of hepatocyte transplantation into various sites such as liver, spleen, pancreas, peritoneal cavity, and sub-renal capsule. The majority of these studies have shown improvement of the biochemical abnormalities post-hepatocyte transplantation. Normalisation was not achieved most probably due to the small number of transplanted hepatocytes, i.e. a limited number of cell could be transplanted at a time. However, the results of these studies suggest that hepatocyte transplantation may become a useful technique for bridging patients to organ transplantation and metabolically support the failing liver and avoiding whole liver transplantation in some metabolic liver diseases (3).

Clinical application of hepatocyte transplantation

The findings obtained from the various animal models of hepatocyte transplantation encouraged human hepatocyte transplantation clinical trials for treatment of patients with:

1) Liver failure

The first attempts were to study the safety, feasibility, and efficacy (2) and it was shown that a decrease in blood ammonia and bilirubin levels in patients with grade III encephalopathy (5). In 1997, Strom and colleagues attempted to use hepatocyte transplantation for end stage liver failure by splenic artery infusion. There were improvements in liver function profile but with no significant improvement
in patient outcome (3). Billr and colleagues (4), reported that five patients with severe acute liver failure (grade III to IV encephalopathy) that had factor V levels less than 0.5 U/ml, and not suitable for orthotopic liver transplantation, underwent intrahepatic (4 patients) and/or intrasplenic (2 patients) hepatocyte transplantation under angiography. There were improvements in brain oedema, and encephalopathy grade, albeit with a 24—72 hour post- hepatocyte transplantation. It must be noted that in this study, cryopreserved hepatocytes were used, and suggest that the cells were of sufficient quality (viability and metabolic activity).

2) Metabolic liver disease

In 1998, Fox and colleagues reported a study in which they treated a 10 year old girl with Crigler-Najjar syndrome type I, and showed that up to 9 months post- hepatocyte transplantation there was sustained stable expression of bilirubin-UDP-glucuronyltransferase activity (6). In 1997, Strun and colleagues treated a 5 year old patient with ornithine transcarbamylase deficiency that led to improvement of ammonia levels but the patient died of pneumonia after 6 weeks (7).

Problems

Some of the problems that need resolving in order to establish hepatocyte transplantion as a feasible routine procedure for treating liver patients include:
- Ideal site of infusion portal vein versus spleen
- Inability to store fresh hepatocytes for longer periods of time, making it difficult to use in emergency situations;
- Number of cells to be transplanted for sufficient long-term correction of function;
- Monitoring of rejection and ongoing need for immunosuppression with its complications.

The obvious solutions for such problems should include: a) improvement of cold storage techniques and also freezing cells to preserve good function on thawing; b) use of growth factors to promote repopulation of the liver, with transplanted cells, with the possible use of stem cells; and c) genetic manipulation of cells for either upregulation of gene expression e.g. enzymatic activity, or rendering them immune-tolerant, hence avoiding immunosuppressive drugs.

Hepatocyte transplantation in SMC

We transplanted allogenic hepatocytes into the liver of an 18-year old boy with glycogen storage disease type 1B (body weight 35 kg, height 1.48 m). Clinical signs include severe hypoglycaemia 3—4 h after eating, increased production of lactic acid, triglycerides, and uric acid, and development of many
hepatic adenomas. He required corn-starch meals every 6 h to maintain blood glucose levels. Hepatocytes, approximating 2% of the liver mass, were infused into his portal vein through a catheter. The child was awake during the cell infusion. After the transplant procedure, the blood glucose levels maintained at 70 to 110 mg/dL and have remained at that level for more than 4 months even though he stopped corn-starch meals. Glucose-6-phosphatase activity was increased to the normal level (5.9~93 nmol/min/mg protein), while no significant change in liver glycogen concentration was noted.

REFERENCES