Hepatocyte Transplantation

Essay

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Historical Review of Hepatocyte Transplantation:

The Road from Whole Liver Transplantation to Hepatocte Transplantation

Like most technological developments in science, hepatocyte transplantation and the technology associated with it were not ideas that just came out of the blue. Immortality has been the oldest dream of humankind and produced the chimera of legend .The chimera has come true in the latter half of the 20th century through the development of advanced surgical techniques and investigation of the rejection reaction and the means to prevent it.

The first experimental trials of liver transplantation as a treatment of irreversible hepatic failure involved auxiliary liver transplantation as reported by *Goodrich et al. (1956*) and orthotopic liver transplantation in dogs as reported by Cannon et al at the same year. In 1963, Starzl et al performed the first clinical liver transplant ,but long-term survival was not achievable due to the status of medicine at that time and lack of effective immunosuppressive techniques.

With the introduction of immunosuppressants such as azathioprine and prednisone, and technical advances in transplant surgery, liver transplantation

was no longer an experimental therapeutic technique and by 1983 had become an established method for treatment of hepatic failure. Organ donation from brain-dead donors, the introduction of new immunosuppressants such as cyclosporin and FK-506, and the development of the Bio-pump have resulted in a marked improvement in the outcome of liver transplantation. The indications for liver transplantation have expanded from end-stage liver disease to metabolic disorders and acute hepatic failure. Shortage of donor organs is now a serious problem in transplantation medicine *(Wiesner,1996).*

While only one liver is available from each donor , the idea of partial liver transplantation came from the fact that the liver has an excellent regenerative capacity. A large number of animal experiments conducted in the 1960s confirmed that partial liver transplantation was technically possible and that transplanted partial liver grafts regenerate to an appropriate volume in proportion to the size of the recipient *(Van der Heyde et al., l966)*. It took approximately 20 years before partial liver transplantation was first applied clinically. In 1984, a reduced-size adult partial liver graft was successfully transplanted into a pediatric patient. Split liver transplantation, which enables the transplantation of two partial liver grafts from one donor liver into two recipients, was first attempted in 1988, and

living-related partial liver transplantation was performed successfully in the following year. The road from whole liver transplantation to partial liver transplantation was thus completed. *(Bismuth et al., 1984)*.

In Japan, where organ donation from brain-dead donors is not permitted, more than 300 living-related liver transplants have been performed and have produced better results than whole liver transplantation. It has already been confirmed that the volume of transplanted partial liver grafts increases in proportion to the growth of pediatric recipients. The concept of partial liver transplantation utilizing liver segments or lobes has lead to the idea of transplantation of small amounts of liver tissue and hepatocytes, or ex vivo use of these materials *(Kawasaki et al.,1992)*

In 1898, Ribbert attempted to transplant liver tissue into lymph nodes and Lubarsch transplanted liver tissue into the lung, liver and kidney. The main objective of liver tissue transplantation in the middle of the 20th century was not functional support for the damaged liver but clarification of the mechanism of regeneration after liver tissue necrosis and restoration of liver tissue after ectopic liver tissue transplantation *(Grisham et al.,1964)*.

Although very rare, ectopic liver originating from pluripotential foregut tissue is sometimes found in the gall bladder, suspensory ligament, umbilicus and lung .which have no connection to the original liver. A congenital ectopic liver has no portal blood supply *(Lieberman, 1966)*. Ectopically transplanted liver tissue has demonstrated histological proliferation of hepatocytes in grafts in subcutaneous tissue or beneath the kidney capsule over a long period (6 months to 1 year). These findings confirm that like congenital ectopic liver, ectopically transplanted liver tissue is able to proliferate without portal blood supply, suggesting an important scientific basis for hepatocyte transplantation .

Professor Eiseman of Colorado University , one of the pioneers of liver surgery and the treatment of acute hepatic failure using extracorporeal xenogenic liver perfusion, referred to the dream of reinfusing cultured liver cells at the 19 symposium of the Colston Research Society held at the University of Bristol in 1967. This was the starting point for hepatocyte transplantation for the treatment of liver disorders *(Eiseman, 1967)*. In 1969, Berry and Friend reported an epoch-making method for isolating hepatocytes using collagenase *(Berry et al., 1996)*.

Many investigators have tried to evaluate the functional support capacity of hepatocyte transplantation using animals with experimentally

induced acute hepatic failure or congenital metabolic disorders.

It has been demonstrated that the creation of an auxiliary liver using hepatocytes is no longer a novel concept. The aim of the work on hepatocyte transplantation was to create a liver mass or secondary liver at ectopic sites using the vigorous regenerative capacity of hepatocytes. The optimum site for hepatocyte transplantation is reported to be the spleen, portal vein, or lung, but differs according to the type of liver disorder and species .We chose the spleen as the site for hepatocyte transplantation as the spleen becomes hypertrophic (500-800g) in the presence of liver cirrhosis and both sufficient and long-term functional support would be possible if the enlarged spleen was occupied by transplanted hepatocytes .

The initial study revealed that the transplanted hepatocytes gradually proliferated so that within 15-27 months after transplantation they occupied 80 % of the spleen. We name this hepatized spleen ***(Kusano et al., 1982)***. Even when 80% of the spleen is occupied by hepatocytes, this is equivalent to only 5-6% of the volume of the recipient whole liver. Not more than 20%) of transplanted hepatocytes remained in the spleen and a large proportion migrated to the recipients liver after intrasplenic transplantation ***(Gupta et al., 1990).***

The volume of hepatocytes that have migrated through the portal vein in the liver is important when evaluating the functional support capacity of hepatocytes transplanted into the spleen. The problem is that it takes too long for the transplanted hepatocytes to proliferate and exhibit sufficient function when the recipient has a normal liver. Moreover, the candidates for hepatocyte transplantation have liver disorders *(Losgen et al., 1984)*.

The volume of hepatocytes necessary to correct impaired liver function depends on the type of the liver disorders. For example, Asonuma et al confirmed by experimental partial liver transplantation in Gunn rats that at least 12% of the whole liver mass was needed for complete correction of serum bilirubin level *(Asonuma et al.,1992)*. This result serves as an index for determining the quantity of hepatocytes necessary for hepatocyte transplantation. For the treatment of chronic hepatic failure, large number of hepatocytes must survive at the ectopic site for a long period.

Attempts have been made to use fetal hepatocytes in place of adult hepatocytes because they have vigorous regenerative capacity and less immunogenic. However, rapid reconstruction of hepatic tissue in the spleen has not yet been achieved using this method *(Ebata et al., 1985)*.

Selective isolation of liver stem cells which have the capacity to differentiate into hepatocytes and biliary ductules is also underway *(Sigal et al.,1992)*. Meanwhile, considerable energy has been directed at establishing non invasive means of increasing the number of surviving hepatocytes at the ectopic sites or in the liver using exogenous growth factors. The use of exogenous growth factors or liver stem cells will help to increase the volume of surviving hepatocytes to levels sufficient for functional support of the impaired liver.

The advantages of cellular transplantation are as follows:(l) simple surgical procedures; (2) the possibility of autologous transplantation not requiring immunosuppressants; (3) the possibility of multiple transplants from a single donor; (4) long-term cryopreservation of cells; (5) easier immunomodulation than with organs and (6) the possibility of gene manipulation. Hepatocyte transplantation does not involve difficult surgical procedures such as careful organ harvesting and vascular anastomosis. Autologous transplantation would enable transplantation of hepatocytes isolated from biopsied liver tissue fragments from the recipients themselves if the proliferation of small number of transplanted hepatocytes could be stimulatedthrough treatment of the recipient with growth factors or gene transfer of growth factors into the hepatocytes before transplantation. Approximately 40% of harvested donor

livers for whole liver transplantation are discarded due to blood type mismatch and are available for hepatocyte transplantation. Long-term cryopreservation of isolated hepatocytes would enable the establishment of a cell bank, multiple transplants of hepatocytes from a single donor, and repeated hepatocyte transplantation. Immunological manipulation of isolated cells is easier than with whole organ *(Balladur et al., 1995)*.

Gene manipulation may prove to be one of the most revolutionary techniques in the field of cell transplantation. Ex vivo gene manipulation is simpler than in vivo gene manipulation in terms of risk and technique. The transfer of genes for galactosidase and antitrypsin into hepatocytes was attempted in the 1990s. In 1995, Grossman et al isolated hepatocytes from the partial liver of patients with familial hypercholesterolemia and performed five intraportal transplants of normalized hepatocytes by ex vivo transfer of an LDL gene using a retrovirus, but the effects were only temporary *(Grossman et al.,1995)*.

Finally, numerous laboratory studies have shown that hepatocyte transplantation may serve as an alternative for patients with life-threatening liver disease. Because of the success of experimental hepatocyte transplantation, institutions have attempted to use this therapy in the clinic for the treatment of a variety of hepatic diseases.

Unfortunately, unequivocal evidence of transplanted human hepatocyte function has been obtained in only one patient with Crigler-Najjar syndrome type I, and, even then, the amount of bilirubin-UGT enzyme activity derived from the transplanted cells was not sufficient to eliminate the patient's eventual need for organ transplantation. A roadmap for improving patient outcome following hepatocyte transplantation can be obtained by a re-examination of previous animal research. A better understanding of the factors that allow hepatocyte integration and survival in the liver and spleen is needed to help reduce the need for repeated cell infusions and multiple donors. Although clinical evidence of hepatocyte function can be used to indicate function of transplanted hepatocytes, definitive histologic evidence is difficult to obtain. In order to assess whether rejection is taking place in a timely fashion, a reliable way of detecting donor hepatocytes will be needed. The most important issue affecting transplantation, however, related to donor availability. Alternatives to the transplantation of allogeneic human hepatocytes include transplantation of hepatocytes derived from fetal, adult or embryonic stem cells, engineered immortalized cells, or hepatocytes derived from other animal species *(American Journal of Transplantation, 2004).*

Preparation of Hepatocyte

Introduction

The liver plays a vital role in the biochemical metabolism of the body and has very complicated functions such as the production of glucose, proteins and lipids, the metabolism of vitamines and hormones, and the secretion and excretion of bile. It also has an excellent capacity for regeneration after partial hepatectomy, a feature not shared by other organs. The detailed study of these biochemical functions and biological characteristics requires a simplified in vitro experimental system using purified liver cells. Furthermore, hepatocyte transplantation and an artificial liver support system using isolated hepatocytes has recently been investigated as treatment of acute and chronic hepatic failure *(Matas et al., 1980)*. A method for isolating intact hepatocytes from the liver is indispensable to these studies.

Various methods have been employed in attempts to isolate liver cells to date. However, with the earlier mechanical and chemical methods it was very difficult to isolate hepatocytes and still maintain sufficient viability. The use ofcollagenase for enzymatic digestion has improved results significantly.

Various isolation techniques in early stage :

1-Mechanical Digestion:
- homogenization *(Palade and Claude 1949)*.
- forcing *(Schneider and Potler 1943)*.
- pipetting *(Longmuir and AP Rees 1956)*
- shaking *(St. Aubin and Bucher 1952).*

2- Chemical Digestion :
- Citrate *(Anderson 1953).*
- EDTA *(Coman 1954)*.
- TPB *(Rappaport and Howz 1966).*

3-Enzymatic treatment:
- trypsin *(St. Aubin and Bucher 1952).*
- pronase *(Roser 1968).*
- lysozyme *(Homines et al. 1970).*
- collagenase + hyaluronidase *(Howard et al. 1974).*

4-CelI Separation :
- Ficoll *(Castagna 1969).*
- Metrizamide *(Munthe-Kaas and Seglen 1974).*

Hepatocyte source and isolation method :

1- Small samples such as biopsied specimens and fetal tissue first shake with 0.5 mM EGTA and then 0.05% collagenase.

2- Small whole liver (cannulation not possible).Needle puncture for collagenase digestion. Cuts then shake with enzyme solution .

3- Whole liver (cannulation possible) .Enzyme perfusion method.
4- Segments and lobes of the liver. Multipuncture perfusion method or enzyme digested method .

Non-enzymatic Methods of Liver Cell Preparation :

In earlier studies, a variety of mechanical methods were used including homogenizers, crushing using stainless steel or silk mesh, pipetting and shaking with glass beads. However, mechanical methods are effective for liver cell transplantation when the liver is pretreated with an enzyme but yields poor results in the absence of such pretreatment *(Plade et al., 1949).*

Chelators were used as a chemical method of cell preparation after it was shown that metal ions, particularly Ca, play an important role in cellular adhesion. The chelators used included citrate, EDTA, EGTA and sodium tetraphenyl boron. Since it was impossible to isolate liver cells satisfactorily using a chelator alone, a combination of chemical and mechanical methods was used. Even with these methods combined, it is difficult to collect a large number of liver cells with sufficient metabolic activity *(Anderson, 1953 and Rappaport, 1966).*