ABSTRACT

Liver transplantation is the only treatment available for acute liver failure. However, mortality rates remain high because of the shortage of donor organs. Indeed up to 20% of patients with acute liver failure may survive without transplantation. In the last two decades, research has focused on the development of alternative or supportive measures to deal with acute liver failure; one of the most studied is hepatocyte transplantation, because it is thought that the function of the liver can only be replaced with a biological substrate characterized by functioning liver cells. Hepatocyte transplantation has been successful in many animal models of acute liver failure, although only several clinical attempts have been made in humans with encouraging but not yet convincing results, mainly because of the lack of a reliable source of live liver cells. Allogenic and xenogenic fresh or cryopreserved hepatocytes have been tested. Recent research has focused on fetal hepatocytes and progenitor liver cells of both hepatic and bone marrow origin. The ability to preserve and bank human hepatocytes would allow pooling of cells from multiple donors to increase the numbers for transplantation. The development of a reliable and large-scale available source of live liver cells would probably have a major impact on the introduction of hepatocyte transplantation in clinical practice.

ORTHOSTOTIC LIVER transplantation, at present the only successful treatment for acute liver failure, shows survival rates ranging from 70% to 85%. However, mortality rates for liver failure remain high because of the shortage of available donor organs. It is thought that the function of the liver can only be replaced by functioning liver cells, thereby requiring the availability of liver tissue from xenogeneic or human sources. Extensive laboratory work and recent clinical studies suggest that hepatocyte transplantation may be a useful bridge to transplantation for selected patients with liver failure. Hepatocyte transplantation has several practical and theoretical advantages over whole liver transplantation. Whereas intact livers can only be transplanted within a short time after procurement, isolated liver cells may be cryopreserved for later use in emergencies. A single donor could potentially provide hepatocytes for several patients, and hepatocyte transplantation should not interfere with subsequent orthotopic liver transplantation. The most reliable sites for hepatocyte engraftment and function are the liver and spleen. The peritoneal cavity may also be a site for transplantation of encapsulated or matrix-attached hepatocytes. Portal vein infusion or injection of hepatocytes into the splenic pulp results in seeding of the transplanted cells into the liver parenchyma. Transplantation of hepatocytes has been shown to significantly improve the survival of animals with both chemically and surgically induced acute liver failure and to prevent the development of intracranial hypertension in pigs with acute ischemic liver failure. Studies using transgenic animals have indicated that massive repopulation of the host liver by engrafted hepatocytes requires that the transplanted cells undergo a proliferative stimulus to which the host hepatocytes cannot respond. The proliferative stimulus may consist of the loss of hepatic mass, such as partial hepatectomy, reperfusion injury, massive apoptosis mediated by Fas-gene transfer, or administration of stimulants of hepatocellular mitosis. Conceptually, hepatocyte transplantation should be especially suitable for treat-
ing acute liver failure because the liver remains architecturally normal and has considerable potential for recovery.

CLINICAL STUDIES

Following the heels of encouraging laboratory results, several centers have instituted clinical hepatocytes transplantation trials. The first from India, described seven patients who each received a single infusion into the peritoneal cavity of fetal hepatocytes that were harvested from 26- to 34-week gestational age fetuses. The overall survival of patients receiving hepatocytes was 43%, as compared with 33% in matched control subjects. All patients with grade III hepatic encephalopathy who received a liver cell transplant survived, whereas matched control subjects who did not receive transplants had a 50% mortality rate. The second report, by Strom et al describes the use of hepatocyte transplantation as a bridge to whole-organ transplantation in five patients. Patients received a mixture of between 107 and 109 freshly isolated and cryopreserved liver cells via splenic arterial perfusion. All five subjects were critically ill with grade IV hepatic encephalopathy and multisystem organ failure. Four patients with illness of equal severity were used as control subjects. All control patients died within 3 days. In contrast, the five liver cell transplant-treated patients maintained normal cerebral perfusion and cardiac stability, with withdrawal of medical support 2 to 10 days before whole-organ transplantation. Blood ammonia levels decreased significantly, and three of the five patients successfully bridged to whole-organ transplant were alive and well at 20 months' follow-up. Bilir et al reported five patients with severe acute liver failure who underwent intrasplenic (n = 4) and/or intrahepatic (n = 2) hepatocyte transplantation. All patients had grade III to IV encephalopathy and factor V levels less than 0.5 U/mL, were ventilator and dialysis dependent, and were not transplant candidates. Three of the five patients who survived 48 hours after hepatocyte transplantation had substantial improvement in encephalopathy scores, arterial ammonia levels, and prothrombin times. Clinical improvement was paralleled by an increase in aminopyrine and caffeine clearances. All patients lived substantially longer after hepatocyte transplantation (12, 28, and 52 days) but eventually died. Postmortem examination showed the presence of transplanted hepatocytes in liver and spleen by light microscopy and fluorescent in situ hybridization (FISH). Although transplanted hepatocytes may have provided clinical benefit, convincing evidence of engraftment and function of the transplanted cells has been difficult to prove; up to 20% of patients with acute liver failure might survive without transplantation. An additional issue potentially explaining the controversial results of hepatocyte transplantation for acute liver failure could be the relatively small numbers of hepatocytes transplanted. Less than optimal liver cell quality and viability after cryopreservation in some of the earlier transplants may have also been a factor. Far better results may be seen in the future if multiple hepatocytes infusions are performed. Further prospective randomized control studies are needed to clarify the outcomes of patients who receive liver cell transplants for acute liver failure.

CELL SOURCES

Fetal Hepatocytes

Fetal hepatocytes have several characteristics that make them potentially suitable as donor cells. In contrast to adult hepatocytes, fetal hepatocytes are thought to be highly proliferative, which may facilitate engraftment and expansion of transplanted cell population.

Bone Marrow-Derived Cells

Several recent articles have investigated the possibility that pluripotent stem cells reside in the bone marrow and can contribute to liver regeneration after noxious insults to the liver. Those studies have been undertaken in both animal models of liver progenitor cell activation and in anecdotal cases of sex-mismatched human liver and bone marrow transplantation analyzing the expression of the Y chromosome in the livers of males transplanted from a female donor and in the hepatic tissue of females with a bone marrow transplant from a male donor. Moreover, Lagasse et al reported the intravenous injection of adult bone marrow cells in the tyrosinemic mouse model and demonstrated rescue of the mouse and restoration of biochemical function of the liver.

Immortalized Cells

Several groups have investigated the use of conditionally immortalized hepatocytes for liver cell transplantation. These cell lines are based on the use of the SV40 large T antigen that can induce temperature-sensitive immortalization through the production of a protein that is active at 33°C, but is conformationally inactivated at 39°C. However, there is some concern that these conditionally immortalized hepatocytes become de-differentiated and would not therefore be suitable for clinical use because of the risk of tumorigenesis. Thereafter, cell lines have been constructed with an additional herpes simplex virus–thymidine kinase so that ganciclovir can be used to destroy any SV40 Tag expressing cells should the control of these cells become unregulated.

Banking of Human Liver Cells

Primary isolated human hepatocytes are the best source of hepatic tissue for liver cell–based therapy. The major drawbacks to the use of primary isolated human hepatocytes are the difficulty to identify human livers suitable for cell isolation and the development of a standardized and centralized method of cell isolation and conservation. Therefore, we have proposed centralization of the process of human hepatocytes isolation in a nationwide banking facility to improve obtainable results, allowing a sufficient amount of human hepatic tissue for clinical and research purposes. Each year, a substan-
tial number of livers (approximately 100 per year in Italy) from heart-beating donors are rejected for transplantation for causes including nonviral cirrhosis, high-grade steatosis, and major parenchymal laceration. Those organs, which would be otherwise lost, have been rescued for human hepatocytes isolation to develop a national based bank of human liver cells for cell therapy and research.

In conclusion, Providing enough cell support, either through transplantation of larger volumes of cells or by harnessing the proliferative ability of the cell transplant and inducing a selective growth advantage of donor cells over host hepatocytes, will be a major clinical step forward for this therapy. Several new studies herald a more promising era, especially in addressing the problem of donor cell shortage for cell transplant programs, such as banking, identification of putative liver stem cells, and in the repopulation of the diseased host liver by donor liver cells.

REFERENCES


