Hepatocyte Transplantation: A Review of Worldwide Clinical Developments and Experiences

Evangelos M Mazaris,[1,3] Christos Th Roussos,[1] Vassilios E Papalois[1,2,3]

Hepatocyte transplantation is a promising treatment for several liver diseases and can also be used as a "bridge" to liver transplantation in cases of liver failure. Although the first animal experiments with this technique began in 1967, it was first applied in humans in 1992. Clearly, the most important advantage of this treatment, compared with liver transplantation, is its simplicity, since no surgery is required for implantation of the cells. Much work has been done over the years to maximize the number of viable hepatocytes that can be isolated from a liver, to prepare the cells prior to transplantation so that the outcome will be more successful, and to identify the optimal site for implantation. We review these efforts along with the worldwide clinical experience with hepatocyte transplantation during the last 13 years.

Key words: *Hepatocyte transplantation, Isolation, Manipulation, Cryopreservation, Therapeutic results*

Hepatic failure is a frequent disease, with chronic liver failure ranking fifth among the mortality factors, as noted by the World Health Organization [1]. It is also well known that there is an 80% mortality rate in patients with acute liver failure (ALF) who develop grade-III or grade-IV encephalopathy. Methods that have been used such as plasmapheresis, hemodialysis (in the case of subsequent renal failure), ultrafiltration, and hemosorption have proven to be ineffective. Liver transplantation remains, until now, the best therapeutic measure in acute as well as chronic liver failure. Because of donor scarcity, transplantable livers are difficult to acquire. Ten donor organs per 1 million patients are required annually in Europe and the United States to satisfy the current demand for livers. Not more than 20% of patients survive up to surgery [1]. In the United States, there has been an increase in waiting times for patients with ALF (those patients are placed at the top of the waiting list) from 3.5 days in the late 1980s to approximately 8 days in the mid-1990s. In fact, with demographic aging and the spread of chronic viral hepatic diseases, there is going to be an increasing number of patients requiring transplantation. Hepatocyte transplantation appears to be either an attractive alternative or adjunct to organ transplantation.

The first enzymatic isolation of hepatocytes from animals was performed in 1967 by Howard and coworkers and was significantly improved in subsequent years [2]. In 1976, the first attempts at hepatocyte transplantation were described involving treatment of inherited metabolic liver disorders in animal models. Further experiments concerning animals as well as humans followed and have produced encouraging results. Still, more research is needed to improve and disseminate this method.

There are certain advantages to hepatocyte transplantation compared with whole liver transplantation: (a) it is technically much easier because cells can simply be injected through vascular catheters, and the technique can even be performed as a day-surgery procedure after radiologic or surgical placement of a portal catheter; (b) it can be repeated relatively easily without any major discomfort for the patient; (c) it is cheaper (projected to be 5%-10% the cost of whole-organ transplantation) [3]; (d) hepatocytes can be cryopreserved and held "on call," ready to be administered

[1]*Division of Surgery, Hammersmith Hospital and* [2]*Transplant Unit St. Mary's Hospital, London, UK*
[3]*Imperial College, London, UK*
Address reprint requests to: *Mr. Vassilios Papalois, Consultant Transplant and General Surgeon and Honorary Senior Lecturer, Transplant Unit, St. Mary's Hospital, Praed Street, London W2 1NY, UK*
Phone: *0044 207 886 1217* ***Fax:*** *0044 207 886 1707*
E-mail: *vassilios.papalois@st-marys.nhs.uk*

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at the clinical team's convenience or during emergencies; (e) there is a potential (under some circumstances such as autologous transplantation of ex vivo genetically modulated hepatocytes) to avoid the need for immunosuppression; (f) it has lower morbidity; (g) several patients could be treated with a single donor liver; and (h) the liver cells could be genetically engineered in vitro to upregulate specific functions. The initial results from studies on hepatocyte transplantation for treating liver diseases have been encouraging.

Indications

Hepatocytes could provide excellent metabolic support in acute and chronic liver failure, as well as definitive treatment for inherited metabolic diseases. Hepatocyte transplantation can also "buy" valuable time acting as a bridge for orthotopic liver transplantation (OLT). Furthermore, it could be used as an excellent vehicle for ex vivo gene therapy [4].

Potential conditions that could be suitable for hepatocyte transplantation are (a) acquired liver disorders, and acute (eg, fulminant) and chronic liver failure (eg, chronic viral hepatitis, cirrhosis) [4]; (b) genetic disorders such as Wilson's disease, a-1 antitrypsin deficiency, erythropoietic protoporphyria, lipidoses (eg, Niemann-Pick disease), X-linked adrenoleukodystrophy, familial amyloidosis, and tyrosinemia type 1 where missing gene products can be substituted by transplanting hepatocytes from allogeneic donors [5]; (c) metabolic deficiency states such as congenital hyperbilirubinemia (eg, Crigler-Najjar syndrome), familial hypercholesterolemia, glycogen storage disease type 1, cystic fibrosis, urea cycle defect (ornithine transcarbamylase deficiency), hyperammonemia syndromes, defects of carbohydrate metabolism, galactosemia, lysosomal storage diseases, and oxalosis in which hepatocytes from donors could produce the missing product or undergo gene manipulation to correct the defect [4]; (d) coagulation disorders such as hemophilia A and factor IX deficiency (hemophilia B), or protein C and S deficiency [4, 5]; and (e) immune disorders such as hereditary angioedema [3]. Studies for several of these conditions have been conducted, both in animals and in humans, and have produced encouraging results.

Contraindications

Contraindications can be divided into those concerning the donor and those concerning the recipient.

It seems that ABO blood type and HLA matching between donor and recipient are not important. Since isolated hepatocytes are being used (which do not contain these antigens), mismatching is not a contraindication.

Livers with hepatic viruses and HIV are, of course, rejected as a source of hepatocytes. Fatty livers could be rejected for hepatocyte donation because of lower cell viability [6]. Also, in fatty livers, inflammatory cytokines are activated, particularly TNF-a, and the microcirculation is damaged making these livers more susceptible to impairment during isolation [6]. Other researchers have discovered that the combination of cold ischemia times greater than 18 hours (or greater than 24 hours according to other studies) and advanced degrees of steatosis have a negative impact on hepatocyte viability as well as on the ability to cryopreserve the cells for later use [2]. Prior portacaval shunt in the recipient could increase the possibility of hepatocyte emboli to the lungs.

Patients with chronic liver disease and portal hypertension must be carefully evaluated before transplantation because there is a risk of portal vein thrombosis, which would further compromise the host liver function [7].

Because of all the advantages mentioned previously, hepatocyte transplantation could be performed in many circumstances where whole-liver transplantation is contraindicated. However, there are clinical scenarios where it is unlikely to be performed, for example, in the case of active sepsis where the patient's clinical status is critical. Also, in metastatic malignancies, cholangiocarcinoma, and AIDS, survival may be too short to justify the risks of transplantation. Finally, psychiatric disorders such as severe intractable depression and active substance abuse that would affect the patient's postoperative quality of life seem to be relative contraindications.

Liver Retrieval

The usual source for hepatocytes is from livers of brain-dead donors that were rejected for OLT owing to anatomic anomalies, biopsy results, or patient medical or social histories [8]. These livers are frequently of marginal quality. Cell transplantation groups are also using discarded liver segments, which, because of vessel damage or recipient size, cannot be used for standard liver transplantation [9]. Hepatocytes obtained from good-quality donor liver segments are the most suitable for hepatocyte

transplantation [2].

Commonly, researchers use livers procured from multiorgan donors. In reports, liver retrieval was performed at the end of retrieval of all organs. Technically, only 2 cm of the common trunk of the portal vein is maintained at the liver hilum for subsequent cannulation. Care is taken to avoid parenchymal laceration. The liver is then flushed with 1 L of organ preservation solution through the portal vein and placed in a sterile environment at 4°C for transfer to the laboratory for hepatocyte isolation [10].

Another method that has been suggested is the retrieval of segment IV (with or without the caudate lobe) that has been used from split-liver procedures. This seems to be a good source of high-quality hepatocytes for cell transplantation. In a standard split-liver procedure in which a liver is allocated between an adult and a pediatric patient, segment IV is allocated to the right lobe, resulting in its relative ischemia in the adult recipient with a potential risk of sepsis or infarction. To overcome this problem, at one center [11], resection of segment IV has become standard practice. The procedure is performed with the removal of 80% of segment IV with the line of resection to the left of the middle hepatic vein, after complete preparation of the left lateral segment and the right lobe. The middle hepatic vein must be preserved to avoid outflow obstruction to the anterior part of segments V and VIII. During the procedure, the vessels on the segment IV side are not ligated but dissected using a mosquito fracture technique. The removal of segment IV adds 30 minutes to the 3 hours required for bench preparation of liver splitting. If segment IV is small, the caudate can also be removed from the right lobe and used as an additional source for isolation of hepatocytes [11].

Researchers also have described other sources for human hepatocytes including tissue obtained from liver resections in patients with colorectal liver metastases. The hepatocytes are isolated from the nontumoral margin; however, there is a possibility of cancer cells being present along with the isolated hepatocytes [2].

Patients with cirrhosis also have been treated with hepatocytes recovered from their own lateral segments [7].

Other researchers have used hepatocytes cultured from a surgical biopsy of one liver segment, and although feasible and safe, no convincing therapeutic effect has been seen [12].

Maximum cold ischemia time

Cold ischemia time is defined as the time between cross-clamping in the donor and the beginning of liver digestion in the laboratory (mean, 14 ± 7 hours according to one study) [10]. Hepatocytes are more resistant to cold ischemia than are vascular endothelial cells and bile duct cells; thus, hepatocytes with high initial viability can be obtained from livers with up to 36 hours of cold ischemia. However, cold ischemia times longer than 18 hours, or according to other researchers, longer than 24 hours, have a negative impact on hepatocyte viability and the ability to cryopreserve the cells for later use [3].

Cold ischemia time affects several key hepatocellular functions such as ATP levels, volume and pH homeostasis, solute transport, and drug metabolism. Furthermore, protein synthesis and mitochondrial function appear to be compromised even after short preservation periods; thus, care needs to be taken not to exceed the maximum cold ischemia time [13].

Hepatocyte isolation (Figure 1)

Hepatocyte isolation began in 1967 with Howard and coworkers, who isolated rat hepatocytes using a combined mechanical/enzymatic technique modified in 1969 by Berry and Friend, which was further developed by Seglen in 1976 to become the *two-step collagenase technique*, as it is known today [2]. If a whole liver is used for hepatocyte isolation, cannulae are placed in the existing major blood vessels of the liver and secured in place by sutures. If liver segments are used, cannulae are placed in any patent vessel on the cut surface and secured by sutures while small vessels are ligated to prevent leakage of any perfusion fluid. The liver is then flushed with heparinized saline (5000 units heparin in 1 L normal saline) to flush any clots from inside the vessels. Afterwards, the cannulae are filled with ice-cold modified Hank's balanced salt solution (HBSS) without Ca^{2+}. The liver is then perfused for a 10-minute single pass with Ca^{2+}-free HBSS, in a cabinet at 37°C to break the binding between cells. The flow rate is adjusted to 30 mL/min/cannula. The tissue is then perfused during a 2-minute single pass with HBSS containing Ca^{2+}. Collagenase is added and perfused in recirculating mode for 30

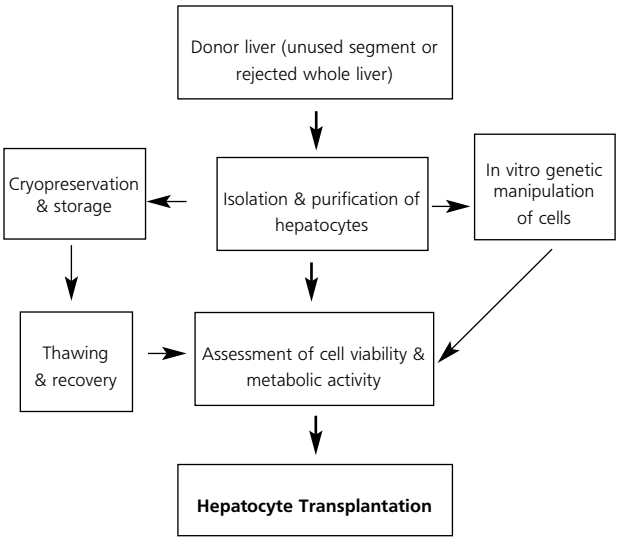

Figure 1. Flow diagram of the preparation of isolated hepatocytes for cell transplantation

minutes to digest the liver, which all this time remains encapsulated. It is then placed in an ice-cold medium, where it is dissected into 2- to 3-mm fragments and filtered through a steel mesh. Hepatocytes are separated by centrifugation (50 g for 5 min) and subsequently washed 3 times with Eagle's modified essential medium at 4°C to purify them [2]. Their viability is assessed by using Trypan blue dye. Researchers in a single center have discovered that hepatic segments deliver a greater yield of high viability hepatocytes when compared with whole livers or larger lobes [11], possibly due to the selection of the healthiest segments, yet more data are required to justify this finding. Baccarani and coworkers have suggested a modification of the above-mentioned method in which adding glycine, alanine, insulin, and fructose to the perfusion fluid in 4 steps leads to protection from ischemia/reperfusion injury and maintenance of the energy status of the hepatic cells, which in turn results in higher postisolation viability [10].

The use of isolated hepatocytes has 2 major drawbacks: a large number is needed to maintain liver function, and adult hepatocytes divide very slowly (unless treated with growth factors) [14]. Although juvenile hepatocytes may have a stronger repopulation potential when compared with adult ones [15], other solutions have been suggested: Fetal hepatic stem cells isolated from interrupted pregnancies may be used (taken with consideration of the strong ethical issues involved) even though some of their functions may differ from adult hepatocytes: they produce alpha-fetoprotein and not albumin, neoglycogenesis begins during the fourth month of fetal development, and others [14]. Oval cells, which are nonparenchymal hepatic cells, can proliferate as well as differentiate into hepatocytes or bile cells, but they must be modified genetically because there is a risk of carcinogenesis [14]. Hematopoietic stem cells, easily recovered from bone marrow of living donors or umbilical cord blood, may have a prehepatic potential [14]. Xenogeneic cells (especially porcine) have been gaining interest by gene modulation of the histocompatibility complex antigens to avoid immune reactions [16]. And pancreatic cells that recently have been identified in the adult pancreas possess hepatocellular functions, but this requires further research [9].

It has been calculated that to support liver failure in adults, 10%-20% of the liver cell mass must be replaced (ie, 10-15 billion cells or 100-150 g hepatocytes) [17].

Pretransplant handling of hepatocytes

Cryopreservation

Researchers have demonstrated that incubating hepatocytes in suspension following isolation, prior to cryopreservation, allows the cells to resist the freezing process and, if cryopreservation is limited to 24 hours, it has significant advantages on albumin production.

Short-term storage (up to 4 days) of hepatocytes can be achieved by maintaining them in modified University of Wisconsin solution at 4°C [2]. During the process of cryopreservation, the aim is to avoid the formation of ice crystals, thus avoiding cell damage and preserving its metabolic function. The procedure involves storage in nitrogen freezers at temperatures below -140°C (researchers have stored hepatocytes for 1 to 8 months at -180°C) [18]. Results concerning storage time have been controversial, with one study demonstrating unaltered viability of hepatocytes preserved for up to 4 years, whereas others have reported decreased viability after long-term cryopreservation [19]. Most protocols use dimethylsulfoxide as a cryopreservant, which has certain advantages when compared with other agents such as glycerol. Moreover, hepatocytes should be frozen at a slow, controlled rate, while the thawing process should be performed quickly at 37°C. Cryopreservation causes a reduction in hepa-

tocyte viability of 25%-35% and a 50% reduction in hepatocyte ability to attach to plastic. More research is required to better understand the requirements of long-term storage [20].

Hepatocyte manipulation

In standard conditions, mature hepatocytes in culture do not usually survive more than 10-14 days and do not proliferate, unless supplied with growth factors [2] such as epithelial growth factor and hepatocyte growth factor [21]. Malignant cells such as hepatoblastoma have been used; however, not only is there a risk of carcinogenesis, but their phenotype is altered as well, making them highly unlikely to produce normal hepatic function [22]. Also, human fetal hepatocytes have undergone telomerase immortalization to proliferate and restore normal liver function [22]. The immortalization of hepatocytes, which would retain normal function, is another way to achieve hepatocyte transplantation, yet the danger of oncogenesis must be overcome first. This can be accomplished by tightly regulating their growth or eliminating the factors resulting in immortalization before their transplantation, and this has been studied in some experimental models [19]. There also is evidence that selective ablation of host hepatocytes is an effective means of inducing proliferation in transplanted cells. For example, carbon-tetrachloride–induced liver injury in experimental models, which damages host hepatocytes in the perivenous areas without affecting transplanted hepatocytes in the periportal areas of the liver lobule, has caused proliferation of those hepatocytes [9]. Further, when host livers in animal models were treated with partial hepatectomy and inhibitors of proliferation, such as retrorsine, the liver was replaced completely with the transplanted cells. Studies even suggest that partial hepatectomy could be replaced by repeated administration of large amounts of triiodothyronine (T3) [9].

Although all of the above can cause systemic toxicity and potential oncogenesis in humans, the concept could possibly be implemented by irradiating (and thus destroying) specific areas of the host liver thereby enhancing proliferation of the transplanted hepatocytes [9]. Similarly, we can use a combination of a mitotic stimulus (such as partial hepatectomy or infusions of growth factors) and a cell-cycle block for the endogenous cells (such as irradiation or DNA-damaging agents) to make hepatocyte transplantation possible.

Hepatocyte-based gene therapy could be used to replace a missing gene product, convey proliferative ability, prevent allograft rejection, or develop xenogeneic hepatocytes in mammalian liver. Ex vivo as well as in vivo gene therapies are being developed to treat genetic liver diseases. In the ex vivo technique, hepatocytes procured from segmental liver resection of the patient are transduced with a therapeutic gene using a viral vector, as well as with genes offering a proliferative advantage over the diseased hepatocytes, while preventing host immune response, then transplanted back into the patient's liver [23]. In the in vivo technique, DNA is injected directly into the peripheral blood circulation, the regional circulation of the liver (ie, portal vein), or into the liver parenchyma [5]. The ex vivo technique could reduce the risk of toxicity and immunologic consequences associated with in vivo gene transfer [24]. A variety of extrahepatic disorders potentially could be treated by hepatocyte gene transfer prior to transplantation, for example genes for coagulation factors, growth hormone, insulin [12], familial hypercholesterolemia, and others. [9]

In conclusion, if gene-transduced hepatocytes could be proliferated in vivo after transplantation with a preparative regimen of liver irradiation, the clinical application of the ex vivo gene therapy could be revived [24].

Recipient preparation

Although immunosuppression has been used in early clinical trials of hepatocyte transplantation and is required for allogenic transplants, there is a potential to minimize its use or even alter the host's immune response to donor liver cells. If hepatocytes are used as a temporary measure for patients awaiting recovery, long-term immunosuppression could be avoided [9]. In fact, hepatocyte transplants have been well-tolerated immunologically, requiring small doses of immunosuppressants, taking into account that in liver graft rejection, the immune response is mainly directed toward the bile duct epithelium and endothelium [3]. In studies, intravenous cyclosporine A as a monotherapy (3 mg/kg initial bolus, titrated to achieve a serum level of 200 to 300 ng/mL) [18] or tacrolimus (12-hour trough levels ~10 μg/L) and a maintenance dose of prednisolone (1 mg/day) [11] have been used as immunosuppressant agents. However, immunosuppression can be avoided by using autologous

hepatocytes retrieved from the patient and transplanted back into him or her after phenotypic correction by introducing a therapeutic gene [3].

Some researchers have proposed protocols for the prophylaxis of infections for the recipient, for example, selective bowel decontamination antibiotic regimens, administration of antifungal agents, and others [25]. Other researchers have suggested preparative irradiation of the recipient liver, thus stimulating the proliferation of the transplanted hepatocytes, unimpeded by the host cells [24].

Transplant technique

Initially, hepatocyte transplantation was attempted, owing to the convenience of easy access both for the transplant technique and for assessing (with biopsies) their posttransplant status, as well as the ability to distinguish their survival and fate when compared with the host hepatocytes in subcutaneous locations, the peritoneal cavity, the inguinal fat pad, skeletal muscles, et cetera. Yet hepatocyte survival at these sites was limited [26] (Table 1).

Multiple laboratory studies suggest that the spleen is a supportive ectopic site for transplanted hepatocytes, although hepatocytes did not survive when transplanted into the splenic arterial bed via angiographic techniques. However, researchers have been able to engraft hepatocytes following direct injection into the splenic pulp as they are entrapped in the sinusoids and vascular spaces, which seems to play an important role in promoting cell engraftment [26]. It is interesting that in a few months, transplanted hepatocytes can proliferate in the spleen and replace approximately 40% of the splenic pulp, a process named *splenic hepatization*. Moreover, they exhibit differentiated synthetic, metabolic, and biliary transport functions, although bile must be drained directly into the blood followed by excretion into the biliary system of the host liver [16, 26].

Hepatocyte transplantation also has been attempted in pulmonary capillaries and alveoli; however, analysis has shown that they are rapidly destroyed in these sites, due to high pressures and immunologic factors [26].

Table 1. Hepatocyte Survival in Ectopic Sites (16)

Superior cell survival	Poor cell survival
Liver	Inguinal or mesenteric fat pad
Spleen	Pulmonary capillaries or parenchyma
Beneath renal capsule	Pancreas
	Salivary gland
	Thymus
	Thyroid
	Peritoneal cavity

Other researchers have found increased survival when hepatocytes are transplanted with an extracellular matrix gel under the kidney capsule [19].

Furthermore, hepatocyte transplantation into the peritoneal cavity (via a peritoneal dialysis catheter) shows diminished cell survival and greater immune responses to gene products [26].

Finally, the most desirable site for transplanted hepatocytes, from a physiological point of view, is the liver itself, especially integration into the liver plates where increases in portal pressure can be avoided. Studies in animals have demonstrated that hepatocyte injection in the portal vein (through a transjugular approach [19] or a portal catheter), its tributaries (splenic, umbilical [11] vein, etc) or even intrasplenically, deposits them in the hepatic sinusoids. There, some are cleared, and approximately 20%-30% of the injected hepatocytes (in animals) rapidly enter in the periportal locations (16-20 hours), which are compatible with entry into the liver lobule. The hepatic sinusoidal bed seems to be capable of sustaining many transplanted hepatocytes (of 10% or even more of the host hepatocyte mass), while cells can be transplanted repeatedly with no significant complications [26]. Consequently, once transplanted, hepatocytes are fully integrated in the liver parenchyma, they retain normal biliary excretory function (which is important for their use in diseases with defective biliary transport and in Wilson's disease).

To summarize, although researchers have suggested that the portal vein is the most useful route for hepatocyte infusion to treat inherited metabolic diseases of structurally normal livers, in massive hepatic necrosis or cirrhosis, the spleen could be an ideal site because hepatocytes infused in the portal vein are translocated to other undesirable vascular beds including the lung (via portosystemic shunts) [3].

Postoperative problems

Some posttransplant problems have been encountered after hepatocyte infusion limiting the success of this technique, including transient hemodynamic instability during intraportal infusion [27]. Adult hepatocytes may form aggregates that can cause portal hypertension or lung emboli. Another disad-

vantage is the possibility of immunologic rejection in allogenic transplantation, which may require chronic immunosuppression. There also are technical difficulties of cryopreserving hepatocytes, which proliferate slowly, as well as the fact that their source is limited from rejected livers in liver transplantation programs [28]. Most of these problems could be eliminated by using stem cells, which are smaller (and thus form small aggregates), are readily cryopreserved, have extensive proliferative properties, and can be isolated from various sources [28].

Another drawback to the whole procedure is a lag time of as much as 48 hours, which is necessary for clinical benefit to occur after hepatocyte transplantation [29].

In addition, there is significant portal hypertension immediately after hepatocyte transplantation, yet it resolves within hours following the procedure, and the vascular anatomy of the liver is also restored to normal [26]. However, there is evidence for ischemic consequences in host hepatocytes in the liver distal to the locations of transplanted hepatocytes in the lobule, but this can be prevented by using vasodilators [26].

Injection of hepatocytes in patients with portal hypertension can cause problems by shunting cells into the lungs through portosystemic communications. Because hepatocytes are larger than the pulmonary capillaries, they become entrapped within the lungs causing pulmonary embolism, pulmonary hypertension, arrhythmias, and heart failure. Studies have shown, however, that despite the injection of hepatocytes in very large numbers, complications are rare, because they are rapidly destroyed and cleared by phagocytes and fail to survive in significant numbers in the long term [16]. Yet, even a temporary deterioration of lung function in a critically ill patient may affect the fragile balance between life and death and should be avoided [25].

In conclusion, there is a risk for carcinogenesis when transplanting genetically modified hepatocytes, especially when they are immortalized. Thus, their growth must be closely regulated, or the factors contributing to immortalization eliminated, before transplantation [19].

Results

A review of the literature identifies 13 studies of 56 hepatocyte transplantations in humans during the last 13 years (Table 2).

Successful treatment of liver diseases with hepatocyte transplantation could be achieved by bridging patients to whole-liver transplantation, by bridging them to recovery of liver function of their native liver, and by engraftment and long-term function of the transplanted hepatocytes [9].

The results of clinical trials have been encouraging with patients developing improvements in encephalopathy, in prothrombin time [18, 25], in ammonia levels, cerebral perfusion, and cardiovascular stability [9]. Another clinical trial [3] not only demonstrated a significant decrease in blood ammonia levels in grade-IV encephalopathy patients, but also, 4 of the 7 patients were successfully bridged to whole-organ transplantation with 1 more recovering completely without transplantation.

In a familial hypercholesterolemia trial, hepatocytes transfected with retroviral genes caused a significant decrease in serum cholesterol levels (up to 20%) for a prolonged period (18 months); however, the trial was abandoned because of potential oncogenicity [5, 9].

In another trial, a patient with Crigler-Najjar syndrome received hepatocyte transplantation resulting in a reduction in total serum bilirubin of 50%, as well as decreasing phototherapy from 12 hours to 6 hours per day, thus improving the patient's quality of life with a posttransplant survival rate of more than 18 months [9].

Another trial attempted to treat urea-cycle defects in an infant. Although initial success resulted in normal protein intake, ammonia levels increased eventually because of graft rejection due to inadequate immunosuppression [9]. Mitry and coworkers transplanted hepatocytes in an ornithine-transcarbamylase–deficient (urea-cycle defect) patient who showed an increase in blood ammonia levels and serum urea while on a normal protein diet, eventually bridging him successfully to partial left lobe orthotopic transplantation [11]. In another patient with the same defect, although there was initial success after hepatocyte transplantation with improvement in biochemical parameters (ie, ammonia levels), the patient eventually died 42 days later of pneumonia [3].

The relative percentage of cells that enter the liver is likely to be significantly less in cirrhosis compared with the percentage entering the normal liver, yet there are data showing that transplanted

Table 2. Results of Hepatocyte Transplantation in Humans

Researchers	Cause of liver disease	Type of hepatocyte transplantation	Number of transplanted hepatocytes	Route of hepatocyte transplantation	Outcome
Mito and coworkers 1992 (30)	Liver cirrhosis (n = 9), Chronic hepatitis (n = 1)	Freshly isolated autologous hepatocytes	$1.7X10^7$- $6X10^8$	Intraportal	Longer survival >10 months was observed in only 1 patient
Habibullah and coworkers 1994 (31)	Fulminant hepatic failure (n = 7)	Pooled blood-group–matched human fetal hepatocytes	$6X10^7$/kg	Intraperitoneal	All patients with grade III encephalopathy (n = 2) survived but with grade IV, only 1 recovered
Grossman and coworkers 1995 (32)	Familial hypercholesterolemia (n = 5)	Hepatocytes transfected with human LDL receptor cDNA using retroviral vectors in vitro before transplantation	$1X10^9$- $3X10^9$	Intraperitoneal	After 18 months reduction of LDL cholesterol, up to 20%
Bilir and coworkers 1996 (33)	Liver cirrhosis, alcohol induced (n = 3)	Not available	Not available	Transjugular intraportal	All alive 4 years later (27)
Soriano and coworkers 1997 (34)	Fulminant hepatic failure (n = 3)	Cryopreserved (duration not described) hepatocytes	$4X10^7$- $4X10^9$	Intraportal	Two patients died although blood ammonia levels were reduced, 1 patient recovered completely
Fox and coworkers 1998 (7)	Crigler-Najjar syndrome 1 (n = 1)	Freshly isolated hepatocytes	$7.5X10^9$	Intraportal	14-fold increase in enzyme activity, serum bilirubin reduction of, and reduction of daily phototherapy by 50%
Strom and coworkers 1999 (3)	Fulminant hepatic failure (n = 11), liver cirrhosis alcohol induced (n = 2), chronic liver failure (hepatitis virus C) (n = 1), ornithine transcarbamylase deficiency (OTC) (n = 1), alpha-1- antitrypsin deficiency (A1AT) (n = 2)	Cryopreserved hepatocytes	$7.5X10^6$- $1.7X10^8$ $2.2X10^7$ (for the A1AT patient)	Intraportal or intrasplenic	From those with fulminant hepatic failure, 6 were bridged to liver transplantation, 1 recovered completely, 4 died. All died with hepatic cirrhosis and chronic liver failure. The patient with OTC died of pneumonia 42 days after the procedure. The patients with A1AT were bridged to liver transplantation
Horslen and coworkers 1999 (22)	OTC deficiency (n = 1)	Freshly isolated hepatocytes	$5.3X10^9$	Intraportal	Temporary relief of hyperammonemia and protein intolerance, which was lost after 11 days due to graft rejection because of insufficient immunosuppression
Bilir and coworkers 2000 (33)	Fulminant hepatic failure (n = 5)	Hepatocytes cryopreserved for 1-8 months	$1.3X10^9$ -$1X10^{10}$	Intraportal	Four patients survived >12 days and then died, but one died in < 72h
Fisher and. coworkers 2000 (35)	Fulminant hepatic failure (n = 1)	Allogeneic hepatocytes	$8.8X10^8$	Intraportal	Patient fully recovered
Muraca and. coworkers 2002 (36)	Glycogen storage disease type I (n = 1)	Allogeneic hepatocyte transplantation	$2X10^9$	Intraportal	9 months after transplantation on only tacrolimus, eats normal diet and can fast for 7 h without experiencing hypoglycemia
Sokal and coworkers 2003 (37)	Infantile Refsum's disease (n = 1)	Fresh isolated hepatocytes and mixed cryopreserved	$2X10^9$	Intrasplenic	Pipecolic acid decreased by 40% and c26:c22 fatty acids ratio decreased by 36% after 18 months
Mitry and. coworkers 2004 (11)	OTC (n = 1)	Not available	$7.3X10^8$	Intraportal through umbilical vein	Improvement in the maintenance of blood ammonia levels and an increase in serum urea (0.5-4.5 mmol/L) while on normal protein diet

hepatocytes can repopulate a cirrhotic liver [7]. In 1996, Bilir and coworkers reported hepatocyte transplantation in 3 patients with alcoholic cirrhosis, all of whom were alive in 2000 [27].

These results suggest that hepatocyte transplantation is a feasible and safe technique that can restore liver function. However, the technique requires continued research and improvement.

Future

As demonstrated by the many studies in the literature, hepatocyte transplantation may become the preferred alternative to whole-organ transplantation. It is less invasive and costs less compared with whole-liver transplantation and also, is not affected by the shortage of donor organs.

It would be helpful to develop rapid tests to assess the quality of livers rejected for whole organ transplantation; this would save time by early exclusion of nonviable hepatocytes [6].

To make use of the limited numbers of organs available for hepatocyte isolation, hepatocyte banks could be established in centers most experienced with the technique [3]. An alternative solution to the scarcity of human livers is hepatocytes from other animal species, which could provide an unlimited supply of predictable quality hepatocytes when needed, although subject to immunologic processes. Preliminary results suggest that this problem can be overcome with conventional immunosuppression [7]. It is also possible that while liver disease could recur following transplantation, nonhuman hepatocytes could be resistant to disease recurrence [7].

New methods are being sought to prevent allograft rejection of transplanted hepatocytes. A "central tolerization" method is to deplete the host's peripheral lymphocytes by injecting antilymphocyte serum and then inoculating in the host's thymus the donor's lymphocytes [3]. A major clinical step forward for this therapy would be to provide enough cell support, either by infusing larger numbers of cells or by enhancing their proliferation by inducing a selective growth advantage of donor cells over host hepatocytes.

Like many other minimally invasive procedures, the prospects of hepatocyte transplantation being performed as an outpatient procedure hold great promise for the future.

References

1. Sukhikh GT, Shtil AA. Transplantation of embryonic hepatocytes. Experimental substantiation of a new approach to the therapy of liver failure. Bull Exp Biol Med 2002; 134(6): 519-524
2. Mitry RR, Hughes RD, Dhawan A. Progress in human hepatocytes: isolation, culture & cryopreservation. Semin Cell Dev Biol 2002; 13: 463-467
3. Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis 1999; 19: 39-48
4. Gupta S, Chowdhury JR. Therapeutic potential of hepatocyte transplantation. Semin Cell Dev Biol 2002; 13(6): 439-446
5. Ott M, Schmidt HJ, Cichon G, Manns MP. Emerging therapies in hepatology: liver-directed gene transfer and hepatocyte transplantation. Cells Tissues Organs 2000; 167: 81-87
6. Mitry RR, Hughes RD, Aw MM, Terry C, Vergani GM, Girlanda R, et al. Human hepatocyte isolation and relationship of cell viability to early graft function. Cell Transplant 2003; 12: 69-74
7. Fox IJ, Chowdhury JR. Hepatocyte transplant. Am J Transplant 2004; 4 (Suppl. 6): 7-13
8. Ostrowska A, Bode DC, Pruss J, Bilir B, Smith GD, Zeisloft S. Investigation of functional and morphological integrity of freshly isolated and cryopreserved human hepatocytes. Cell Tissue Bank 2000; 1: 55-68
9. Allen KJ, Soriano HE. Liver cell transplantation: The road to clinical application. J Lab Clin Med 2001; 138: 298-312
10. Baccarani U, Sanna A, Cariani A, Sainz-Barriga M, Adani GL, Zambito AM, et al. Isolation of human hepatocytes from livers rejected for liver transplantation on a national basis: results of a 2-year experience. Liver Transpl 2003; 9(5): 506-512
11. Mitry RR, Dhawan A, Hughes RD, Bansal S, Lehec S, Terry C, et al. One liver, three recipients: Segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. Transplantation 2004; 77(10): 1614-1616
12. Guha C, Roy-Chowdhury N, Jauregui H, Roy-Chowdhury J. Hepatocyte-based gene therapy. J Hepatobiliary Pancreat Surg 2001; 8: 51-57
13. Kukan M, Haddad SP. Role of hepatocytes and bile duct cells in preservation-reperfusion injury of liver grafts. Liver Transpl 2001; 7(5): 381-400
14. Feldmann G. Liver transplantation of hepatic stem cells: potential use for treating liver diseases. Cell Biol Toxicol 2001; 17: 77-85
15. Walldorf J, Aurich H, Cai H, Runge D, Christ B, Strom SC, Fleig WE. Expanding hepatocytes in vitro before cell transplantation: Donor age-dependent proliferative capacity of cultured human hepatocytes. Scand J Gastroenterol 2004; 39: 584-593
16. Malhi H, Gupta S. Hepatocyte transplantation: new horizons and challenges. J Hepatobiliary Pancreat Surg 2001; 8: 40-50
17. Haghighi KS, Woon WW, Akhter J, Marr PJ, Bolton E, Riordan S, Morris DL. A new source of hepatocytes for transplantation. Transplant Proc 2004; 36: 2466-2468
18. Bilir BM, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J, et al. Hepatocyte transplantation in acute liver failure. Liver Transpl 2000; 6(1): 32-40
19. Ohashi K, Park F, Kay MA. Hepatocyte transplantation: clinical and experimental application. J Mol Med 2001; 79: 617-630
20. Lloyd TDR, Orr S, Skett P, Berry DP, Dennison AR. Cryopreservation of hepatocytes: A review of current methods for banking. Cell Tissue Bank 2003; 4: 3-15
21. Michalopoulos GK, De Frances MC. Liver regeneration. Science 1997; 276: 60-66
22. Horslen SP, Fox IJ. Hepatocyte transplantation. Transplantation 2004; 77(10): 1481-1486
23. Sundback C, Vacanti J. Alternatives to liver transplantation: From hepatocyte transplantation to tissue-engineered organs. Gastroenterology 2000; 118(2): 438-442
24. Guha C, Deb NJ, Sappal BS, Ghosh SS, Roy-Chowdhury N, Roy-Chowdhury J. Amplification of engrafted hepatocytes by preparative manipulation of the host liver. Artif Organs 2001; 25(7): 522-528
25. Rust C, Gores G. Hepatocyte transplantation in acute liver failure: A new therapeutic option for the next millennium? Liver Transpl 2000; 6(1): 41-43
26. Gupta S, Bhargava KK, Novikof P. Mechanisms of engraftment during liver repopulation with hepatocyte transplantation. Semin Liver Dis 1999; 19(1): 15-26
27. Kobayashi N, Noguchi H, Watanabe T, Matsumura T, Totsugawa T, Fujiwara T, et al. Establishment of a tightly regulated human cell line for the development of hepatocyte transplantation. Hum Cell 2000; 13(1): 7-13
28. Susick R, Moss N, Kubota H, Lecluyse E, Hamilton G, Luntz T, et al. Hepatic progenitors and strategies for liver cell therapies. Ann N Y Acad Sci 2001; 944: 398-419
29. Chan C, Berthiaume F, Nath BD, Tilles AW, Toner M, Yarmush L. Hepatic tissue engineering for adjunct and temporary liver support: Critical technologies. Liver Transpl 2004; 10(11): 1331-1342
30. Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc 1992; 24: 3052-3053
31. Habibullah C, Syed I, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation 1994; 27: 951-952
32. Grossman M, Rader D, Muller D, Kolansky D, Kozarsky K, Clark BJ, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med 1995; 1: 1148-1154
33. Bilir B, Durham J, Krystal J, Karrer F, Kumpe D, Ostrowska A, et al. Transjugular intra-portal transplantation of cryopreserved human hepatocytes in a patient with acute liver failure. Hepatology 1996;

24(4): 308A
34. Soriano H, Wood R, Kang D, Ozaki C, Finegold M, Bishoff F, et al. Hepatocellular transplantation in children with fulminant liver failure. Hepatology 1997; 26(4): 239A
35. Fisher RA, Bu D, Thompson M, Tisnado J, Prasad U, Sterling R, et al. Defining hepatocellular chimerism in a liver failure patient bridged with hepatocyte infusion. Transplantation 2000; 69: 303-307
36. Muraca M, Gerunda G, Neri D, Vilei M, Granato A, Feltracco P, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet 2002; 359(26): 317-318
37. Sokal E, Smets F, Bourgois A, Van Maldergem L, Buts J, Reding R, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety and metabolic follow-up. Transplantation 2003; 76(4): 735-738