CIRUGÍA ESPAÑOLA

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Review article

Hepatic cell transplantation: A new therapy in liver diseases

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ARTICLE INFORMATION

Article history:
Received March 13, 2009
Accepted March 12, 2010

Keywords:
Liver transplantation
Hepatocyte transplantation
Hepatocyte
Inborn errors of liver metabolism
Liver failure

ABSTRACT

Liver transplantation has been remarkably effective in the treatment in patients with end-stage liver disease. However, disparity between solid-organ supply and increased demand is the greatest limitation, resulting in longer waiting times and increase in mortality of transplant recipients. This situation creates the need to seek alternatives to orthotopic liver transplantation.Hepatocyte transplantation or liver cell transplantation has been proposed as the best method to support patients.

The procedure consists of transplanting individual cells to a recipient organ in sufficient quantity to survive and restore the function. The capacity of hepatic regeneration is the biological basis of hepatocyte transplantation. This therapeutic option is an experimental procedure in some patients with inborn errors of metabolism, fulminant hepatic failure and acute and chronic liver failure, as a bridge to orthotopic liver transplantation.

In the Hospital La Fe of Valencia, we performed the first hepatocyte trasplantation in Spain creating a new research work on transplant program.

Trasplante celular hepático: un nuevo tratamiento en las enfermedades hepáticas

Palabras clave:
Trasplante hepático
Trasplante celular hepático
Hepatocitos
Errores congénitos del metabolismo
Fallo hepático

RESUMEN

El trasplante hepático es el único tratamiento efectivo existente para las enfermedades hepáticas en fase terminal. La desproporción entre la demanda y la oferta de órganos constituye su principal limitación y plantea la necesidad de buscar alternativas al trasplante hepático.

El trasplante celular hepático o trasplante de hepatocitos humanos constituye, en el momento actual, la mejor opción terapéutica sustitutiva. Consiste en trasplantar hepatocitos humanos totalmente diferenciados a un órgano receptor, en cantidad suficiente para que éstos sobrevivan y restauren la función hepática normal, basándose en la capacidad de regeneración hepática. Este tratamiento está en fase clinicoexperimental, y se ha realizado

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en pacientes con errores congénitos del metabolismo, fallo hepático fulminante y fallo hepático agudo o crónico como puente al trasplante convencional.

En el Hospital La Fe de Valencia hemos puesto en marcha y llevado a cabo el primer trasplante celular hepático en España, por tanto, esto abre una nueva línea de trabajo dentro del Programa de Trasplante Hepático.

Introduction

The treatment of choice for terminal-stage liver diseases is a liver transplant (LT). The principal limitation of the procedure lies in the imbalance between patients requiring a transplant and organ donors, creating a long waiting list and increasing morbidity and mortality. This creates the need for finding strategies that could serve as partial or total alternatives to LT.

Among the proposed alternatives, the most promising, based on results obtained, is liver cell transplantation (LCT), or human hepatocyte transplantation. The results from this treatment indicate that it could be a very useful technique, as long as viable, high-quality, metabolically functioning human hepatocytes are available.[1-31]

The recent inrush in the field of "regenerative medicine" has opened a new realm of therapeutic options for diseases caused by the cellular deterioration of an organ, and in which a whole-organ transplant is not feasible.

Cellular treatment centres on the hepatocyte as the main deficient cell type. Only totally differentiated cells are used, hepatocytes, which are capable of developing hepatic functions based on the regenerative capacity of the liver.[32-34]

The use of this procedure as an alternative to conventional LT has been researched during the last 30 years. The first studies were performed by Howard in 1967,[35] using animal models with metabolic liver diseases, which resulted in improved biochemistry following LCT.[36] Subsequently, experimental models have been described for acute[37-39] and chronic[40,41] liver failure and hepatic metabolic disorders.[42,43] Clinical tests have been performed in patients with fulminant hepatic failure (FHF)[1,7,9,11,22-24,44,45] or chronic/acute liver disorders as a bridge to LT[5,7,16,23,25] and in children with congenital metabolic errors.[4,6,8,10,12,13,17-21]

Liver cell transplantation as a therapeutic alternative to conventional liver transplants

In spite of the broadened selection criteria for donors, organs remain scarce, necessitating the search for other options. Among these are artificial liver support systems, xenotransplantation, *ex vivo* and *in vivo* gene treatment, tissue engineering, and LCT.

Currently, LCT constitutes the best alternative to orthotopic LT (OLT), given the results obtained.

The alternatives to LT are applied so that LT is avoided, reducing the number of patients on the waiting list. They are also used to maintain liver function until the LT, and in the case of FHF, conserve liver function long enough to allow hepatic regeneration and recuperate liver function without having to resort to LT.[2,7,44]

LCT is performed at the Liver Cell Therapy Unit at the Hospital La Fe, Valencia, whose primary objective is to bring basic research into clinical practice. This Unit's installations are accredited according to the European guidelines EN-ISO 14644 for clean rooms and adjoining premises. The clinical activity here is focused on subsidiary patients of LCT, obtaining organ grafts that have been rejected for LT thanks to a tight collaboration with Spain's National Transplant Organisation. The large-scale preparation of cells is performed under good manufacturing practices.

Liver cell transplantation. Concept and objectives

LCT consists of transplanting completely differentiated human hepatocytes into a receiving organ in large enough quantities to ensure survival of these cells and a restoration of normal function.

The primary objective of LCT is to recuperate and maintain liver function until an adequate donor organ is available, to temporarily substitute hepatocyte function, and in the cases of metabolic diseases, restore the enzymatic deficiency which causes the disease, either as a bridge until LT is possible, or until achieving sufficient recuperation of enzymatic function.

LCT is based on the extraordinary capacity for regeneration presented by liver cells as a response to any kind of cellular damage. Under normal conditions, the liver is in a state of proliferative rest and remains able to divide cells in response to any type of toxic or viral aggression, or surgical resection. Partial hepatectomy is the experimental model that best evidences the capacity for hepatic regeneration that is not accompanied by hepatocellular lesions.[46]

If the intention of liver cell transplantation is hepatic regeneration, the cell type initially used must be a mature hepatocyte, since its replicative capacity enables it to rapidly restore the lost cell population.

Liver regeneration does not depend on progenitor cells, but rather it originates because of mature cell proliferation.[46]

Essentially, the LCT process consists of three phases: hepatocyte isolation, followed by the preparation of cell suspensions, and finally implantation into the recipient.

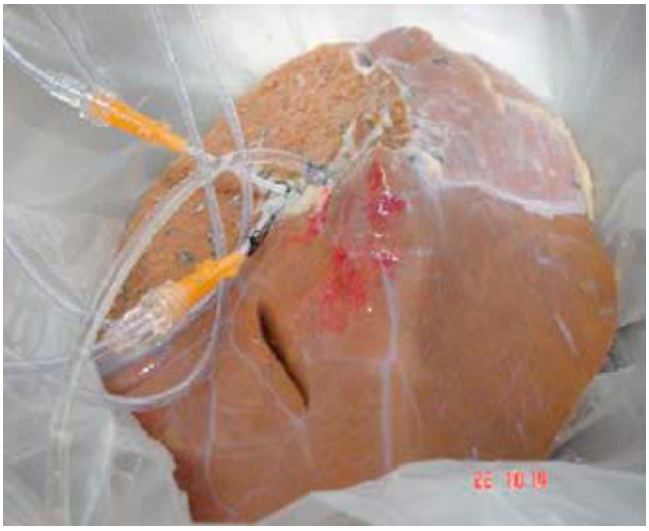

Figure 1 – Obtaining hepatocytes from a split.

Following isolation, the cells can be frozen and stored for later use.

Hepatocytes are obtained from donor organs that were deemed unfit for transplantation for various causes.[1,8-11] Hepatocytes are isolated through a liver perfusion using collagenase[37,47,48] (Figure 1). Approximately 5%-10% of the liver cell mass is transplanted by infusing fresh or cryogenically preserved cells through a portal or splenic vessel. It has a viability of at least 50% and various infusions are performed.[1-5]

Indications and results from liver cell transplantation

Congenital metabolic errors constitute the main indication for LCT in current practice. Other indications include treating FHF patients as a bridge until a solid organ transplant becomes available; patients with chronic hepatopathy on the LT waiting list that present severe decompensation, as a symptomatic treatment until the transplant is available; and in severe liver failure following a major liver resection[1-30] (Table 1).

In our unit, children with congenital metabolic disorders are classified according to whether or not they have indications of LT, the clinical situation of the disease, response to treatment, and quality of life.

Congenital metabolic errors

This is the condition in which LCT has shown the greatest clinical success[1,4,6,8,10,12,13,17-21,31] (Table 2).

Strom et al at the University of Pittsburgh reviewed the results from 30 patients at 6 medical centres, summarising the results from LCT treatment on 20 children with this disease (Table 2): 11 of these procedures served as bridges until organ transplantation, 10 of which received OLT and one of which received an auxiliary transplant.[1,13,16,18,19]

Table 1 – Indications of liver cell transplantation

Genetic disorders that imply liver dysfunction
- Alpha-1-antitrypsin deficiency
- Wilson's disease
- Type I tyrosinemia
- Erythropoietic protoporphyria
- Lipoidosis (Niemann Pick)
- X chromosome-linked adrenoleukodystrophy
- Familial amyloidosis

Enzymatic deficits with curable liver dysfunction
- Congenital hyperbilirubinemia (Crigler-Najjar)
- Familial hypercholesterolemia
- Cystic fibrosis
- Type Ia glycogenosis
- Urea cycle defect (deficit in ornithine transcarbamylase)
- Refsum's disease
- Hyperammonemia
- Carbohydrate metabolism defects
- Galactosaemia
- Lysosomal diseases
- Oxalosis

Coagulation disorders A and B haemophilia, C and S protein deficit, and factor VII deficit
Immunological alterations: hereditary angioedema
Liver failure following major liver resection
Acute/fulminant liver failure
Acute or chronic decompensated cirrhosis

One of the first LCT was performed in 1998[10] on a ten-year-old girl with Crigler-Najjar syndrome, resulting in stable enzymatic activity nine months after the procedure; she later received an OLT. Another eight-year-old girl with the same metabolic disorder received nine hepatocyte infusions, which reduced serum bilirubin (Bb) levels and the need for phototherapy; a transplant was performed 20 months later.[15] In another Crigler-Najjar[13] case, total Bb levels reduced following the LCT and a liver graft was performed 146 days after the LCT procedure. Recently, two new cases of LCT treatment for type I Crigler-Najjar syndrome have been published.[31]

Alterations in the urea cycle (OTC) constitute one of the most frequent indications of LCT. In medical literature, five of the seven patients with this metabolic disorder that received LCT also later received an OLT.

In Belgium, Sokal et al performed LCT on two children with altered urea cycles and observed a reduction in ammonium levels and metabolic stabilisation with psychomotor improvement up until the LT several months later.[17,21]

LCT were performed at King's College on two patients with factor VII deficit with satisfactory results, allowing for an 80% drop in requirements for exogenous factor VIIa. OLT was performed on both patients, at seven and eight months, respectively.[12,20] At this same centre, LCT performed on two children with progressive familial intrahepatic cholestasis did not produce favourable results due to the presence of fibrosis, and finally an LT was performed.[20]

Two patients with type I glycogenosis showed satisfactory development following an LCT (Table 2), with a partial enzymatic

Table 2 – Liver cell transplantation in congenital metabolic diseases

Disease	Age, months	No. of cells, $\times 10^9$	Follow-up	Development	Reference
Altered urea cycle (OTC)	B-3 m	9.4	Protein tolerance	LT 3 m	27
	B-1 m	1.9	↓NH_4 ↑urea	Auxiliary LT 6 m	4
	14-18	2.4	↓NH_4 ↑urea	LT 6 m	30
	42-47	3.5	↓NH_4 ↑urea	LT 18 m	29
	60	1	↓NH_4 ↑urea	↓NH_4 and M 43 d	3
	3.5 w	4	↓NH_4	LT 6 m	18
	25	3	↓NH_4	–	Lee 05, PC
Crigler-Najjar disease	120	7.5	↓60% Bb	LT 3.5 y	10
	96	6	↓40% Bb	LT 20 m	15
	108	7.5	↓50% Bb	LT 4.6 m	13
	18	–	↓50% Bb	LT 8 m	20
	36	–	↓30% Bb	Under supervision	20
	9	2.2	↓50% Bb×5 m	Lost	–
	40	4.3	↓40% Bb×6 m	–	28
	96	1.5	↓30% Bb		Allen 04, PC
	108	6.1	↓ Bb	LT 6 m	31
	12	2.6	↓ Bb	LT	31
Type I glycogenosis	564	2	↓30% triglycerides	Persisted at 18 m	8
Type I glycogenosis	216	9	N-glucose-6-phosphatase	Under supervision	26
FHC	–	0.2	↓20% cholesterol		6
Autotransplant	336	1.1	↓20% cholesterol, LDL	Persisted at 18 m	6
	144	1.3	<effect		6
	84	1	6% cholesterol, LDL	Persisted at 19 m	6
	492	3.2	<effect		6
	132	1.5	20% cholesterol, LDL	Persisted at 7 m	
Factor VII deficit	3	1.1	↓80% nec fac VII 6 m	LT 7 m	12
	35	2.2	↓80% nec fac VII 3 m	LT 8 m	12
	–		↓80% nec fac VII 3 m	Under control	20
Refsum's disease	48	2	↓40% metabolites	Persisted at 18 m	21
PFIC		0.3	No clear benefit	LT 5 m	20
		0.3	No clear benefit	LT 14 m	20

↓ indicates decrease; ↑: increase; B, birth; Bb, bilirubin; d, days; fac, factor; FHC, familial hypercholesterolemia; LDL, low-density lipoprotein; LT, liver transplantation; m, months; M, mortality following transplant; nec, necessities; NH_4, ammonium; OTC, ornithine transcarbamylase deficiency; PC, personal communication; PFIC, progressive familial intrahepatic cholestasis; w, weeks; y, years.

correction, a significant correction in hypoglycaemias, and a 30%-40% reduction in triglycerides.[8,26]

At the Hospital La Fe in Valencia, the first LCT was performed on May 20 2008, on a 12-year-old girl with urea cycle deficiency, neurological alteration and cognitive deficit, severe dietary restrictions, and multiple episodes of decompensation. She had to be hospitalised recurrently during the three months prior to the LCT. Two portal vein infusions of cryopreserved cells were administered with one-week intervals, through a Port-a-Cath placed in the inferior mesenteric vein branch. The cells were taken from an organ that was rejected for whole transplantation due to ischemia.

5.2×10^8 and 3.5×10^8 cryopreserved cells were administered in the first and second infusions, with 74% and 73% cell viability, respectively, following thawing. The initial results showed a reduction in ammonium levels and an increase in the Quick index. Protein ingestion was restored to 1mg/kg/day. The patient presented with fever due to a clinical decompensation with a new increase in ammonium levels and deterioration in liver function. Haemocultures detected the presence of *Aspergillus*, which did not respond to antibiotic treatment and caused the death of the patient due to a massive aspergillosis 15 days following the LCT.

The second patient that we treated using LCT was a nine-month-old girl with type I Crigler-Najjar syndrome with continuous home phototherapy. We placed a Port-a-Cath by laparoscopy for the cell infusion in a branch of the superior mesenteric vein. At present, seven portal cell infusions have been administered over three months, with a total of 26.63×10^8 cryopreserved cells. The infusions, from various donors in the same blood group, with a viability of 47%-75% following thawing, produced a decrease in serum Bb levels of 28-15 mg/dl (Figure 2).

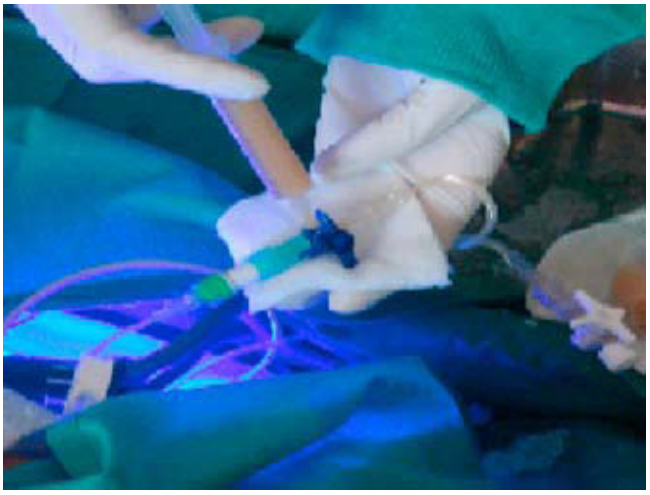

Figure 2 – Hepatocyte infusion of a liver cell transplantation in a patient with Crigler-Najjar syndrome.

In both cases, we performed an antibiotic prophylaxis before the infusion and administered corticosteroid boluses during the procedure. Heart rate, blood pressure, and temperature all remained within normal ranges. The Doppler ultrasound performed on the liver before, during, and after the infusion detected no changes in portal pressure, and in both cases, immunosuppression with tachrolimus was started after the cell infusions. No complications were detected following LCT in either case. Only the first patient presented a skin rash that disappeared following pharmacological treatment.

We are currently using LCT to treat a six-year-old girl with type I glycogenosis, who presents with frequent episodes of hypoglycaemia with hospital admissions and very high levels of triglycerides, cholesterol, and uric acid.

Fulminant hepatic failure

In spite of the priority status given these patients on the LT list, they present an elevated mortality rate. In this situation, LCT is used as a bridge until conventional transplantation is feasible, and helps to maintain the patient alive until an organ is made available, or until achieving liver regeneration[1,2,9,44,49] (Table 3).

The benefit of LCT in these patients is difficult to demonstrate given the various parameters that influence the evolution of liver function, the small number of cases, and the short wait until OLT. LCT appears to improve survival, grade of encephalopathy, and ammonium levels.[1,9,11,15,16]

Three patients with FHF had a spontaneous recovery following LCT, and thus no longer required LT, although this could be attributed to a remission of the disease. Between 1% and 5% of the hepatic mass is infused into the patient through the splenic artery or portal vein to the liver,[1,9] delivering 10^7-10^{10} hepatocytes.

In the first case of LCT used to treat FHF, with grade III-IV encephalopathy, Habibullah infused $6x10^7$ foetal hepatocytes into the peritoneal cavity, and confirmed increased survival as compared to the control group (48% vs 33%). Encephalopathy improved 48 hours after LCT, and Bb and ammonium levels were reduced.[11]

Bilir performed LCT on five patients with acute liver failure, with grade III and IV encephalopathy, with no indications of LT. They infused between 1% and 10% of the hepatic mass in three patients with toxic aetiology and in two patients with viral aetiology at the splenic or hepatic level. Following LCT, the grade of encephalopathy, ammonium levels, and prothrombin time improved in three patients.

Strom performed LCT in four patients with FHF (Table 3) with grade IV encephalopathy and organ failure, using this procedure as a bridge for LT.[1]

In most publications, the main reason for the low efficiency of transplanted hepatocytes resides in the small number of infused cells. Meanwhile, in FHF, hepatic necrosis produces toxic substances that are capable of inhibiting regeneration of the transplanted hepatocytes.

Acute and chronic liver disease and decompensated cirrhosis

Acute and chronic liver failure is more frequent in adults than in children; in both cases, LCT is used as bridge until OLT. It could improve residual function and quality of life in patients with decompensated cirrhosis, and may also improve survival[16] (Table 4).

In 1993, Mito performed the first LCT with autogenous hepatocytes on ten patients with hepatic cirrhosis, infusing the cells into the spleen and using the left lateral segment of the recipient for cell isolation.[50,51]

Chronic liver diseases imply a loss in the hepatic parenchyma without the presence of compensatory proliferation, and this tissue is replaced by fibrous tissue, fat accumulations, and cirrhosis. Under these conditions, LCT presents limitations: the implanted hepatocytes, upon finding themselves in an adverse environment, are incapable of regenerating the parenchyma, necessitating alternative implantation points, such as the spleen.[1,9,16,23,24]

LCT performed on these patients in the United States[5,16] infused the hepatocytes, whether fresh or cryopreserved, through the splenic artery or portal vein; between 10^7 and 10^{10} cells were infused up to a maximum of 5% of the normal liver volume. The results showed that this procedure served as a bridge until OLT in 8 patients; 2 patients recovered completely no longer needing LT, and 16 patients died. When indicated, LCT produced an improvement in patients with cirrhosis with normalisation of encephalopathy and anuria.[4,16]

LCT was performed on 3 children of 3 weeks, 3 months, and 6 months of age, with idiopathic, cryptogenic,[23] and septic[1] liver cirrhosis, respectively. Ammonium levels reduced and encephalopathy improved in the first two patients, with a complete recovery 6 and 7 days, respectively, after OLT. The third patient died seven days after the LCT.

In two patients with alpha-1-antitrypsin deficit, the primary objective was to recuperate the deficient enzyme with normalisation of its plasma activity, which could indicate the existence of a liver repopulation by the transplanted

Table 3 – Liver cell transplantation in fulminant hepatic failure

Indication	Age, years	No. of cells $\times 10^9$	Infusion point	Encephalopathy	Development	Reference
HBV	37	0.88	Spleen	II	$\downarrow NH_4$, CR	16
HBV	43	0.72	Portal	IV	LT 1 d	16
HBV	28	0.16	Spleen	IV	$\downarrow NH_4$ and LT 3 d	1
HBV	65	30	Portal+spleen	III	$\downarrow NH_4$, ↓encephalopathy, and M 52 d	9
HBV	40	0.06	Intraperitoneal	IV	M 13 d	
HBV+lymphoma	64	6.6	Portal	II	$\downarrow NH_4$, ↓encephalopathy, and M 7 d	
TPN/sepsis	0.6	5.2	Spleen	IV	$\downarrow NH_4$, M 7 d	1
Idiopathic	3	4	Portal	III	CR	23
Idiopathic	48	0.75	Portal	IV	M 1 d	Fisher, NP
Idiopathic	0.35	0.18	Portal	I	LT 1 d	54
Idiopathic	5	1.4	Portal	IV	$\downarrow NH_4$ and LT 4d	54
Idiopathic	23	0.46	Spleen	III	LT, M 5 d	16
Idiopathic	8	0.06	Intraperitoneal	III	CR	11
Drugs*	15	0.4	Portal	IV	$\downarrow NH_4$, M 2 d	
Drugs*	32	1.3	Spleen	IV	$\downarrow NH_4$, ↓encephalopathy, M 14 d	9
Drugs*	13		Spleen		M 4 d	1
Drugs*	26	1.2	Spleen	IV	LT 2 d	16
Drugs*	27	0.28	Spleen	IV	$\downarrow NH_4$, LT 10 d	1
Drugs*	13	0.29	Spleen	IV	M 4 d	1
HS+drugs*	37	0.12	Spleen	IV	$\downarrow NH_4$, ↓encephalopathy, and M 5 d	16
HS	29	10	Portal+spleen	IV	M 18 h	9
NSAID+alcohol	35	10	Spleen	IV	$\downarrow NH_4$, ↓encephalopathy, M 20 d	
Trisegmentectomy	69	0.53	Spleen	IV	M 2 d	16
Amanita poisoning	64	4.9	Portal	IV	M 1 d	54
Acute fatty liver and pregnancy	26	0.3	–	IV	CR	9

↓ indicates decrease; CR, complete recovery; d, days; h, hours; HBV, hepatitis B virus; HS, herpes simplex; LT, liver transplant; M, mortality following the transplant; NH4, ammonium; NP, not published; TPN, total parenteral nutrition; NSAID, nonsteroidal anti-inflammatory.
*Acetaminophen and dilantin.

hepatocytes. One of these patients received an OLT three days after the LCT.[1]

At the Hospital La Fe, we performed LCT on a 47-year-old male on the waiting list for a second liver transplant due to a recurrence of C-virus in the implanted organ. He was admitted to hospital due to a sudden decompensation that occurred during the wait and for a grade IV encephalopathy that required assisted breathing. Four infusions were administered with cryopreserved cells into the splenic artery. The cells came from a graft that had been rejected for transplantation due to ischemia and were administered percutaneously through the femoral artery and implanted into the spleen. A total of $2.2x10^8$ cells were infused, with a viability that ranged from 51% to 68% following thawing (Figure 2). Antibiotic prophylaxis was administered before infusion and corticosteroid boluses during the process. We also administered immunosuppression protocols with corticosteroids and tachrolimus.

The clinical and analytical evolution was favourable; the patient presented significant improvement in encephalopathy, allowing extubation; figures for urea, creatinine, Bb, and ammonium levels also reduced, allowing for the patient to ingest orally. No compatible donor organ was made available during this time, and the patient went on to present a high digestive haemorrhage secondary to the rupture of oesophageal varices, causing fulminant death of the patient at 15 days post-LCT.

Clinical evaluation of patients following a liver cell transplantation

The various LCT working groups have all related a clinical and analytical improvement observed in reduced ammonium levels and prothrombin time, improved encephalopathy, and an increase in the deficient enzymes in patients with metabolic disorders.[1,2,4,9,10,12,13,16-19]

However, it is not clear whether this improvement is a result of the LCT, considering that a low quantity of hepatocytes have been transplanted in many of these patients. Nor is there any exact understanding as to the duration of procedure's efficacy, and it is the clinical evolution of the patient which indicates whether new cell infusions are necessary. It is also important to note that the hepatocytes' capacity to be implanted into the recipient tissue is low (under 10%), which can be insufficient to achieve a clear benefit.[2]

The most commonly used technique for determining hepatocyte survival in the recipient liver is a post-transplant liver biopsy. This detects the donor hepatocytes or an

Table 4 – Acute and chronic decompensated cirrhosis

Indication	Age, years	No. of cells, $\times 10^7$	Infusion point	Encephalopathy	Development	Reference
AC	46			II-III	M 50 d	16
AC	62		Spleen	II-III	M 33 d	16
AC	–			–	Alive	16
AC	–			–	Alive	16
AC	–			–	Alive	16
Dα-1-AT	52	2.2	Spleen	IV	↓NH_4 and LT 2 d	1
Dα-1-AT	0.3			I	LT 4 d	16
HCV	40	0.8	Spleen	IV	↓Encephalopathy, M 5 d	1
Dysfunction following LT	56	200	Portal	–	M 0 d	25
Cryptogenic	3w	70	Portal	–	↓NH_4, LT 1 w	23
Fibrosis	3 m	50	Portal	–	↓Encephalopathy, LT 6 w	23
HCV	62	0.6	Spleen	III	↓NH_4	54

↓ indicates decrease; AC, alcoholic cirrhosis; d: days; Da-1-AT, deficit in alpha-1-antitrypsin; HCV, hepatitis C virus; LT, liver transplantation; m, months; M, mortality following transplantation; NH4, ammonium; w: weeks.

increase in deficient enzyme activity.[9-11,13] Furthermore, histological evidence is difficult to come by.

Transplanted hepatocytes have been observed in the liver and spleen upon autopsy.[9] Other authors advocate using nuclear medicine techniques to detect infused hepatocytes.

Three to six months after the infusion, the metabolic improvements that were obtained begin to weaken, which could be considered as a rejection or delayed cell death of the transplanted hepatocytes; repeating cell infusions could prevent this loss of function.

Compatibility and immunosuppression

As in conventional transplantations, ABO compatibility must be ensured, and an immunosuppressive regimen must be applied in order avoid rejection, since hepatocytes can be rejected in the same way as other transplanted organs.[52]

Steroid boluses are necessary during LCT infusion, as well as the use of immunosuppressive drugs, and the most commonly used regimen is a double treatment with corticosteroids and calcineurin inhibitors in similar dosages as those used in LT.

However, it is difficult to detect cell rejection, since the rejection markers used in LT to assess the donor organ can not be applied to LCT.

Corticosteroid boluses were administered to the three patients who received hepatocyte transplantation, (one bolus per patient), once each of the infusions had been applied. In all cases, immunosuppressive treatment was later started with tachrolimus.

Advantages of liver cell transplantation

The main advantage of using the procedure as opposed to LT is that it is not a major surgical procedure. It has lower morbidity and mortality and lower costs, and is a much less invasive procedure.[2,5,8]

LCT also offers the possibility of using cells from one single donor for several recipients, maximising the donor resources.

The procedure may also be performed in a semi-programmed fashion given that the cells can be cryopreserved.[8,19,20]

In LCT, the transplanted cells are functional and capable of maintaining liver functions until a whole organ is obtained. In some cases, the facilitation of liver regeneration constitutes a cure and serves as an alternative for LT.

The results published by different working groups with respect to the application of LCT to certain congenital metabolic disorders indicate that this procedure is an efficient bridge treatment and at times even an alternative for OLT.[2,18,20,21,25]

Limitations of liver cell transplantation

The main limitation of LCT lies in the lack of adequate viable hepatocyte sources. Currently, the only hepatocyte sources are organs that have been rejected for implantation, the resulting tissue from liver reductions, and the tissue left over from split transplants. Fatty liver disease, the main motive for rejecting a donated organ for OLT, is not considered an optimal source for hepatocyte isolation.[47,53,54]

Another obstacle for LCT resides in the rapid elimination of transplanted hepatocytes by recipient macrophages. Controlling transplanted hepatocytes poses problems due to the lack of knowledge on the mechanisms that allow infused hepatocytes integrate into the liver or spleen and their survival. No studies have been performed on patients to evaluate the immune response triggered by the transplanted hepatocytes, their repopulation in the recipient, the causes of viability loss, or cell rejection, nor do we know which drugs to

use to avoid these issues. Furthermore, we do not know the exact clinical efficacy of LCT.

Most of the articles published have been isolated clinical cases, and no controlled clinical trials have been performed on humans, making the scientific evidence available on the subject weak.

The newest areas of research observe methods used to enhance the regenerative capacity of transplanted hepatocytes, such as the use of epithelial growth factors, hormones (such as insulin or T3), and oval cells.

Complications in liver cell transplantation

There are less complications associated with LCT than LT; the most frequent being infection and thrombosis.[23,25]

Increased portal pressure has been described following the first cell infusions, which then returns to normal. Portal vein thrombosis, portal hypertension, and even pulmonary embolism can be produced.[49]

Infiltration of the splenic artery can cause infection, embolism, and at times, pulmonary complications.[12] Patients with acute or chronic liver failure can present sepsis, viremia, and multiple organ failure, although the relation that these complications have with LCT is undefined.[9]

The need for continuous immunosuppressive treatment creates problems in maintaining the levels within the therapeutic range.[13]

Conflict of interest

The authors affirm that they have no conflicts of interest.

REFERENCES

1. Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation. 1997;63:559-69.
2. Smets F, Najimi M, Sokal EM. Cell transplantation in the treatment of liver diseases. Pediatr Transplant. 2008;12:6-13.
3. Strom SC, Fisher RA, Rubinstein WS, Barranger JA, Towbin RB, Charron M, et al. Transplantation of human hepatocytes. Transplant Proc. 1997;29:2103-6.
4. Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for metabolic disorders, experience at King's College Hospital and review of literature. Acta Gastroenterol Belg. 2005;68:457-60.
5. Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. Transplantation. 2006;82:441-9.
6. Grossman M, Raper SE, Kozarsky K, Stein EA, Engelhardt JF, Muller D, et al. Successful ex vivo gene therapy directed to liver in a patient with familial hypercholesterolaemia. Nat Genet. 1994;6:335-41.
7. Riordan SM, Williams R. Extracorporeal support and hepatocyte transplantation in acute liver failure and cirrhosis. J Gastroenterol Hepatol. 1999;14:757-70. Review.
8. Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet. 2002;359:317-8.
9. Bilir BM, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J, et al. Hepatocyte transplantation in acute liver failure. Liver Transplantation. 2000;6:32-40.
10. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998;338:1422-6.
11. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation. 1994;58:951-2.
12. Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, et al. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation. 2004;78:1812-4.
13. Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant. 2005;14:151-7.
14. Navarro-Álvarez N, Soto-Gutiérrez A, Kobayashi N. Hepatocyte transplantation: a step forward. Curr Opin Organ Transplant. 2008;12:652-8.
15. Darwish AA, Sokal E, Stéphenne X, Najimi M, De Ville de Goyet J, Reding R. Permanent access to the portal system for cellular transplantation using an implantable port device. Liver Transpl. 2004;10:1213-5.
16. Strom SC, Roy-Chowdhury J, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Sem Liver Dis. 1999;19:39-48.
17. Sokal EM. Liver transplantation for inborn errors of liver metabolism. J Inherit Metab Dis. 2006;29:426-30.
18. Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics. 2003;111:1262-7.
19. Stepehenne X, Najimi M, Smets F, Reding R, De Ville de Goyet J, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Am J Transplant. 2005;5:2058-61.
20. Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis. 2006; 29:431-5.
21. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. Transplantation. 2003;76:735-8.
22. Bilir B, Durham JD, Kristal J. Transjugular intraportal transplantation of cryopreserved human hepatocytes in a patient with acute liver failure. Hepatology. 1996;24:728.
23. Soriano HE, Wood RP, Kang DC. Hepatocellular transplantation (HCT) in children with fulminant liver failure (FLF). Hepatology. 1997;26:239A.
24. Khan AA, Habeeb A, Parveen N, Naseem B, Babu RP, Capoor AK, et al. Peritoneal transplantation of human fetal hepatocytes for the treatment of acute fatty liver of pregnancy: a case report. Trop Gastroenterol. 2004;25:141-3.
25. Baccarani U, Adani GL, Sanna A, Avellini C, Sainz-Barriga M, Lorenzin D, et al. Portal vein trombosis after intraportal hepatocytes transplantation in a liver transplant recipient. Transpl International. 2005;18:750-4.
26. Lee KW, Lee JH, Shin SW, Kim SJ, Joh JW, Lee DH, et al. Hepatocyte transplantation for glycogen storage disease type Ib. Cell Transplant. 2007;16:629-37.
27. Allen KJ, Soriano HE. Liver cell transplantation: the road to clinical application. J Lab Clin Med. 2001;138:298-312.
28. Hughes RD, Mitry RR, Dhawan A. Hepatocyte transplantation for metabolic liver disease: UK experience. J R Soc Med. 2005;98:341-5.
29. Stéphenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity

after liver cell transplantation for argininosuccinate lyase deficiency. Gastroenterology. 2006;130:1317-23.
30. Stéphenne X, Najimi M, Smets F, Sokal EM, Reding R, Goyet JV. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Case Report Am J Trans. 2005;5:2058-61.
31. Lysy PA, Najimi M, Stephenne X, Bourgois A, Smets F, Sokal E. Liver cell transplantation for Crigler Najjar syndrome type I: update and perspectives. World J Gastroenterol. 2008;14: 3464-70.
32. Zimmermann A. Regulation of liver regeneration. Nephrol Dial. 2004;19 Suppl 4:iv6-10.
33. Ito M, Nagata H, Miyakawa S, Fox IJ. Review of hepatocyte transplantation. J Hepatobiliary Pancreat Surg. 2008;6:34-8.
34. Tabú R. Liver regeneration: from myth to mechanism. Nature Reviews Mol Cel. 2004;5:836-47.
35. Howard RB, Christensen AK, Gibbs FA, Pessch LA. The enzimatic preparation of isolated intact parenchymal cells from rat liver. J Cell Biol. 1967;35:675-8.
36. Eiseman B. Treatment of liver failure. In: Read AE 8Ed. Colston Papers No. 19. The Liver. London: Butterworths; 1967. p. 279-85.
37. Serralta A, Donato MT, Orbis F, Castell JV, Mir J, Gómez-Lechón MJ. Functionality of cultured human hepatocytes from elective samples, cadaveric grafts and hepatectomies. Toxicol In Vitro. 2003;17:769-74.
38. Sutherland DE, Numata M, Matas AJ, Simmons RL, Najarian JS. Hepatocellular transplantation in acute liver failure. Surgery. 1977;82:124-32.
39. Ahmad TA, Fujioka H, Eguchi S, Yanaga K, Kamohara Y, Furui J, et al. Long-term effect of hepatocyte transplantation on fulminant hepatic failure in rats. Hepatogastroenterology. 2003;50:467-71.
40. Ahmad TA, Eguchi S, Yanaga K, Miyamoto S, Kamohara Y, Fujioka H, et al. Role of intrasplenic hepatocyte transplantation in improving survival and liver regeneration after hepatic resection in cirrhotic rats. Cell Transplant. 2002;11:399-402.
41. Kobayashi N, Ito M, Nakamura J, Cai J, Gao C, Hammel JM, et al. Hepatocyte transplantation in rats with decompensated cirrhosis. Hepatology. 2000;31:851-7.
42. Mito M, Kusano M, Sawa M. Hepatocyte transplantation for hepatic failure. Transplantat Reviews. 1993;73:5-43.
43. De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology. 2000;119:1720-30.
44. Palmes D, Qayumi AK, Spiegel HU. Liver bridging techniques in the treatment of acute liver failure. J Invest Surg. 2000;13: 299-311.
45. Bilir BM, Guenette D, Ostrowska A. Percutaneous hepatocyte transplantation (PHT) in liver failure. Hepatology. 1997;26:494.
46. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006;43(2 Suppl 1):S45-53.
47. Mitry RR, Hughes RD, Dhawan A. Progress in human hepatocytes: isolation, culture & cryopreservation. Semin Cell Dev Biol. 2002;13:463-7.
48. Gómez-Lechón MJ, Donato T, Ponsoda X, Fabra R, Trullenque R, Castell JV. Isolation, culture and use of human hepatocytes in drug research. In: Vitro methods in pharmaceitical research. London: Academic Press; 1997; p. 129-53.
49. Ott M, Barthold M, Alexandrova K. Clinical applications of human hepatocytes isolated under CGMP conditions. 40th annual meeting of the European Association for the study of the liver. J Hepatol. 2005;42(2 Suppl):1-283. AB 176.
50. Mito M, Kusano M. Hepatocyte transplantation in man. Cell Transplant. 1993;2:65-74.
51. Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc. 1992;24:3052-3.
52. Bumgardner GL, Gao D, Li J, Bickerstaff A, Orosz CG. MHC-identical heart and hepatocyte allografts evoke opposite immune responses within the same host. Transplantation. 2002;74:855-64.
53. Donato MT, Lahoz A, Jiménez N, Pérez G, Serralta A, Mir J, et al. Potential impact of steatosis on cytochrome P450 enzymes of human hepatocytes isolated from fatty liver grafts. Drug Metab Dispos. 2006;34:1556-62.
54. Sterling RK, Fisher RA. Liver transplantation: living donor, hepatocyte, and xenotransplantation. In: Gish R, editor. Current future treatment therapies for liver disease. Clinics in liver disease. Philadelphia: WB Saunders; 2001.