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Review

Hepatocyte transplantation in humans: past accomplishments and future perspectives

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SUMMARY

The rapidly increasing frequency of liver transplantation and subsequent shortage of donor organs has caused investigators to focus on hepatocyte transplantation (HCTx), as an alternative therapeutic method for patients with various hepatic diseases. To date, experimental data show that engrafted hepatocytes can survive in ectopic sites for a long period of time retaining their functionality to some extent. Despite these encouraging findings in animal environment, the recent clinical implementation of this novel method has made little progress. This review aims to summarize the currently existing experience on the clinical HCTx and to designate its potential perspectives and limitations in various fields, such as cell procurement and preservation, transplantation technique, transplantation immunology and genetical manipulations of the transplantable hepatocytes.

Key words: Clinical hepatocyte transplantation, humans

Hepatocyte transplantation (HCTx) has been investigated in experimental animals for nearly two decades, as potential treatment for acute and chronic liver failure (due to any cause) and as "gene therapy" for various inborn metabolic disorders. Although, several encouraging findings have been obtained in animal environment, the recent clinical implementation of this novel method has made little progress. The aim of this review is to summarize the currently existing experience (Table 1) about the human HCTx and to designate its potential perspectives or limitations.

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Hepatocyte origin and procurement. The first point to be considered for clinical HCTx is the origin and the number of the transplantable cells. To date, in all clinical trials, human hepatocytes have been used.[1-9] Their isolation from a segment of either the autologous liver or a donated organ is carried out successfully, in terms both of cells number and viability. Two distinct enzymatic perfusion techniques, for liver digestion, are currently established: portal vein cannulation and multi-puncture method. The former consists in catheter insertion into the umbilical vein (portal branch) and is indicated for hepatocyte isolation from liver lobes.[11] The latter is accomplished with multiple punctures, directly to the hepatic parenchyma, using thick needles with side holes and is indicated in cases of small liver segments.[1,11] In future, two potential sources for hepatocytes procurement may be proven useful. The first includes donated organs (150-200 livers per year in the USA, according to general estimates), which are found to be unsuitable for whole or partial liver transplantation.[12] The second could be the so-called "marginal cadaver livers", presenting a high risk of primary nonfunction, whose cells, however, are considered of doubted functionality.[13] Moreover, HCTx offers the obvious advantage of multiple transplants from a single donor. The number of cells needed to support the missing liver function has not been precisely determined, but a discrepancy seems to exist between expected and provided liver support, by the transplanted cells. Thus, experimental transplantation of hepatocytes, corresponding to about 2% of total liver mass, has resulted in native liver recovery in cases of reversible acute hepatic insufficiency and corrected metabolic disorders in various animal models.[12]

Embryonic cell transplantation. Human fetus could be

Table 1. An up-to-date review of clinical experience on Hepatocyte Transplantation. *(Abbreviations: Tx: Transplantation, HCTx: Hepatocyte Transplantation, ALF: Acute Liver Failure, LDL: Low Density Lipoprotein, OTC: Ornithine Transcarbamylase, HSV: Herpes Simplex Virus, A1AT: a-1-antithrypsin, TPN: Total Parenteral Nutrition)*

Publication Year	Author (Ref.No)	Number and Age of Patients	Indication	Type and Site of HCTx	Results
1992	Mito[1]	10 patients (46-78 y.o.)	Liver cirrhosis (9) and chronic hepatitis(1) of Child score A(6), B(1) and C(3).	Tx of autologous hepatocytes in the spleen.	Detection of engrafted cells with scintigram, up to 11 months after HCTx.
1994	Habibulah [2]	7 patients (8-32 y.o.)	ALF of undetermined etiology, with encephalopathy of grade III(2), IVa(1) and IVb(4)	Tx of human fetal hepatocytes in the peritoneal cavity.	Significant improvement of survival rate (43% in transplant group vs 33% in control group) in patients with grade III and IVa encephalopathy.
1995	Grossman[3]	5 patients (7-41 y.o.)	Familial homozygous hypercholesterolemia	Intraportal tx of autologous genetically manipulated hepatocytes.	LDL levels significantly decreased in 3 patients (60%). Transgene expression has been assessed, up to 4 months after the HCTx.
1996	Reyes[4]	1 patient (2.5 y.o.)	OTC deficiency	Repeated intraportal tx of allogeneic hepatocytes.	Whereas detectable OTC activity was present after the first attempt of HCTx, the patient died of pneumonia, 5 weeks later.
1996	Bilir[5]	1 patient (29 y.o.)	ALF due to HSV II infection.	Injection of allogeneic, cryopreserved hepatocytes, via the jugular vein.	The patient died, 18 hrs after the HCTx, because of irreversible cerebral edema. However, donor cells have been detected in host's liver and lungs.
1997	Strom[6,7]	6 patients (6mo.-52 y.o.)	Viral(1) and drug induced(2) ALF, chronic hepatitis C(1), A1AT deficiency (1), TPN /sepsis(1)	Allotransplantation of freshly isolated and cryopreserved hepatocytes in the spleen (5) and the native liver (1).	The high mortality (50%), within first week following the HCTx, was due to endocranial hemorrhage. In 3 patients, HCTx served as a bridge to successful liver Tx.
1998	Fox[8]	1 patient (10 y.o.)	Criggler-Najjar syndrome (type I)	Intraportal allotransplantation of freshly isolated hepatocytes.	Obvious clinical improvement and significant decrease of hyperbilirubinemia. Conjugated bilirubin has been detected in the bile, up to 6 months after the HCTx.
2000	Bilir[9]	5 patients	ALF with grade III and IV encephalopathy.	Allotransplantation of cryopreserved hepatocytes in spleen and/or native liver.	All patients died within 52 days after HCTx, despite the observed temporary clinical and biochemical improvement.

an alternative source of hepatocytes. Fetal cells have a vigorous regenerative capacity and would be well tolerated by an allogeneic host.[14] While serious ethical concerns, arising from the possible therapeutic utility of human embryos remain unresolved, Habibulah et al have successfully transplanted fetal hepatocytes, in children

and young adults with acute liver failure.[2] Furthermore, the possibility for selective isolation of liver stem cells, which can be subsequently differentiated to adult hepatocytes, is currently under meticulous investigation, being the most viable option for the near future.[15]

Perspectives for xenotransplantation. Transplanting hepatocytes of animal origin (mainly swine) is another, less promising, perspective of the HCTx. Despite the obvious immunological problems, the fear of transmissing a viral or bacterial zoonosis, which may adversely impact the immunocompromised recepient's health, delays the clinical implementation of xenogeneic HCTx.[16]

Perspectives for autotransplantation. Theoretically, the use of autologous cells is the "golden standard", for a successful HCTx in humans. In 1967, Prof. Eiseman, one of the pioneers in the development of various hepatic support systems, predicted "a reinfusion of tissue culture liver cells".[17] Today, his vision is about to become a reality. Autologous cells have been isolated, in sufficient number and high viability, from liver segments of various size and biopsy specimens.[18] These cells, when transplanted, could be stimulated to proliferate in their ectopic site, by potent growth factors (such as Hepatocyte Stimulating Substance-HSS), which may be either exogenously administered[19] or producted in vivo by transgenic hepatocytes.[20]

Use of cryopreserved, cultured or conditionally immortalized cells. In contrast to whole liver, hepatocytes can be preserved for long period. With the recent advances of cryopreservation methods, the establishment of a "cell bank" is no more a novel concept.[21] Moreover, hepatocytes would be a renewable source, since they retain the capacity of proliferating in culture. Two major approaches towards this direction are currently under development. The first aims to the clonal expansion of differentiated hepatocytes in vitro, induced by special growth factors.[22] The second tempts to create conditionally immortalized cells, with genetic manipulations. A viral vector (simian virus – SV40) carrying a temperature sensitive antigen gene has been tried, in the early studies by Schumacher et al.[23] When viral DNA is integrated in the cellular genome, hepatocyte replication is induced (especially in presence of growth factors), provided that temperature remains constantly lower than 33C. A serious concern, with this approach, is the possibility of neoplastic transformation of the genetically manipulated cells. Thus, the clinical application of this method relies primarily on maintaining sufficient safeguards, which must offer a high level of assurance, that replicating transgenic cells would not escape regular growth control, in vivo.[24]

New trends in hepatocyte transplantation immunology. Since the early era of experimental HCTx, it was expected that liver cells could be transplanted across immunologic barriers, without immunosuppressive therapy of the recepient.[25] To date, in all published clinical trials of HCTx, the applicated immunosuppression protocols have been identical to those used for whole organ transplantation, at the respective medical centers.[4-8] However, according to experimental data "transplantation without need for immunosuppression" could be carried out through methods of either immune regulation or immune isolation of the transplantable cells. For the modulation of cellular antigenicity, there might be excellent prospects through hepatocyte culture (a field in which our group is actively involved[26,27]) and UVB-irradiation of the cells before their engraftment.[28] Furthermore, transplantation of hepatocytes from transgenic animals, which express human histocompatibility antigens and are not recognized as "non self" by the host, may be proven useful for the same purpose.[29] For the immunoisolation of allogeneic cells, an innovative technique for encapsulating hepatocytes into semipermeable membranes has been evaluated in the laboratory setting, leading to encouraging results.[30]

Hepatocytes transplantation and gene therapy. Nevertheless, the most attractive perspective of human HCTx seems to be the gene therapy of inherited metabolic diseases. To date, one case of familial homozygous hypercholesterolemia and one case of Criggler-Najaar syndrom successfully treated with HCTx, have been reported.[3,8] However, there still exist technical limitations mainly concerning the regulation and the maintenance of an adequate gene expression rate in the engrafted hepatocytes.[31] Inevitably, in future, the optimization of this method will enlarge the spectrum of potential applications, which may include various inborn disorders, such as ornithine transcarbamylase-deficiency (OTCD), syndroms of unconjugated hyperbilirubinemia, Wilson's disease, inherited tyrosinemia, hemophilia B and even the hepatocellular carcinoma.[32]

Choice of the appropriate anatomical site for hepatocyte engraftment. From technical view, HCTx is much simpler than whole or partial liver transplantation. It is a relatively non invasive technique and complicated vascular anastomoses are not required. To date, three different anatomical sites for cell engraftment have been tested, in the clinical trials of HCTx: native liver,[3-9] peritoneal cavity[2] and spleen.[3,4,6-8] Hepatocytes infusion in the native liver has been carried out via the portal or the jugular vein. Despite its theoretical advantages, in ex-

perimental practice HCTx into the liver has been followed by serious complications, such as portal hypertension, hepatic necrosis and cirrhosis.[12] On the other hand, peritoneal cavity permits transplantation of an adequate number of cells by minimally invasive means, but it has been proven unable to ensure prompt engraftment of the translanted cells, which is absolutely necessary in cases of fulminant liver failure.[33] Thus, spleen seems to be the most appropriate site for HCTx. Due to its structure, splenic red pulp entraps the injected hepatocytes, offering prompt perfusion and the possibility of beneficial interactions with cells of mesenchymal origin.[34]

Today, it is difficult to predict which of the parallel roads, mentioned above, will successfully lead to the clinical integration of HCTx. Even if we accept that, the two major events in the evolution of this method have been the enzymatic liver digestion and the development of gene manipulation techniques, we can hardly preview the next big step. However, it is worthy to remind that, according to Prof. Mito, an inspired investigator in this field, the control of stem cells proliferation and differentiation, will lead HCTx to its "third era of technological revolution".[14]

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