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REVIEW

Liver cell transplantation for Crigler-Najjar syndrome type Ⅰ: Update and perspectives

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Abstract

Liver cell transplantation is an attractive technique to treat liver-based inborn errors of metabolism. The feasibility and efficacy of the procedure has been demonstrated, leading to medium term partial metabolic control of various diseases. Crigler-Najjar is the paradigm of such diseases in that the host liver is lacking one function with an otherwise normal parenchyma. The patient is at permanent risk for irreversible brain damage. The goal of liver cell transplantation is to reduce serum bilirubin levels within safe limits and to alleviate phototherapy requirements to improve quality of life. Preliminary data on Gunn rats, the rodent model of the disease, were encouraging and have led to successful clinical trials. Herein we report on two additional patients and describe the current limits of the technique in terms of durability of the response as compared to alternative therapeutic procedures. We discuss the future developments of the technique and new emerging perspectives.

Key words: Hepatocyte transplantation; Cell therapy; Inborn error of metabolism; Crigler-Najjar; Liver regeneration; Animal models

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INTRODUCTION

Crigler-Najjar (CN) syndrome is the paradigm of an inborn error of liver metabolism affecting the function of one enzyme, the 1A1 isoform of the bilirubin-uridine diphosphate glucuronosyltransferase (UGT1A1)[1]. The parenchyma and thousands of other metabolic functions are normal, but the patient is at risk for severe neurological complications. Quality of life is deeply impaired, requiring phototherapy up to 12 h daily with efficacy lessening with ageing (probably due to unfavorable body surface/weight ratio and to increased skin thickness and pigmentation). Orthotopic liver transplantation (OLT) is a curative for the disorder[2,3], but seems disproportionate to correct one single missing enzymatic function in an otherwise normal liver. Patients and physicians are often reluctant to undertake such an irreversible procedure and are seeking less invasive alternative options. Indeed, up to 15% of OLT patients require re-transplantation, and progressive fibrosis of the graft is a subject of concern at long term[4].

Auxiliary liver transplantation (ALT) is another curative approach that has the advantage of being reversible. However, ALT remains associated with major pitfalls. In addition to being an invasive surgical procedure, the technique is difficult mainly because of perilous anastomosis that can hamper the venous in- or outflow and can lead to graft atrophy/ischemia or vascular thrombosis. Another complication is the small-for-size liver syndrome, defined as liver impairment, following inadequate liver mass replacement[5]. The diagnosis of rejection is difficult because of minimal enzyme elevation.

Table 1 Representative liver cell transplantation experiments in the Gunn rat model

Donor cells	Injection site	Hepatic injury	Outcome	Cell tracking	References
50×10^6 free or encapsulated congeneic Hc	Peritoneum	None	34.8% serum bilirubin reduction with encapsulated Hc *vs* 13.5% with free Hc at 1 mo	Light and electron microscopy	22
10×10^6 syngeneic Hc	Liver	Hepatectomy	Significant reduction of serum bilirubin up to 4 wk Apparition of conjugates in bile	ND	24
10×10^6 congeneic Hc	Spleen	None	Significant reduction of serum bilirubin up to 12 mo Apparition of bile conjugates at 4 mo	ND	27
2-20 $\times 10^6$ congeneic Hc	Portal vein	Right portal vein ligation	Significant reduction of serum bilirubin when injury with 2×10^6 Hc or with 20×10^6 without injury up to 30 d Conjugates in bile after 10 d	UGT1A1 activity, WB, PCR for *ugt1* gene	28
5×10^6 congeneic Hc	Spleen	Hepatic irradiation ± Hepatectomy	Normalization of serum bilirubin only with combined injury Conjugates in bile detected up to 5 mo	UGT1A1 activity, WB, IHC	29
10×10^6 congeneic Hc	Spleen	Hepatic irradiation ± FasL-induced apoptosis	Normalization of serum bilirubin up to 160 d Conjugates in bile at 150 d Estimation of repopulation at 52 ± 15% when combined injury	UGT1A1 activity, WB, IHC	31
40×10^6 fetal or adult syngeneic Hc	Spleen	Retrorsine + Triiodothyronine	Significant reduction of serum bilirubin (+ conjugates in bile) up to 90 d (no difference between fetal and adult cells)	PCNA	32

Hc: Hepatocyte; IHC: Immunohistochemistry; PCNA: Proliferating cell nuclear antigen; WB: Western blot.

Successful long-term results were recently obtained with gene therapy in Gunn rats[6,7]. This technique was described to depend on vector serotypes and allowed a reduction of serum bilirubin up to 64% after one year[8]. Globally, this technique is still facing with anti-UGT1A1 antibody production in the host organism, impeding the perpetuation of the metabolic effect[9]. Although encouraging, *ex vivo* gene transfer and cell injection is closely related to the quality of cell preparation[10,11] and has not been documented in CN patients.

Other experimental protocols have been described, such as tin-mesoporphyrin treatment, for which feasibility has been demonstrated in two 17 year old patients[12], or treatment with chimeric oligonucleotides that allowed a significant reduction of serum bilirubin in Gunn rats for up to 11 mo[13].

Since the princeps report by Fox *et al*[14], liver cell therapy (LCT) appeared as a new alternative treatment, which is intermediate between whole organ transplantation and gene therapy. Cells can be infused safely in the diseased liver, and are expected to bring sufficient enzyme activity to restore bilirubin metabolism, setting the patients within safer metabolic limits and improving quality of life. LCT has been shown to be able to restore metabolic function not only in CN patients[15], but also in disorders of ammonium metabolism[16,17], glucose metabolism[18], clotting factor deficiencies[19], and even complex enzyme systems such as Refsum disease[20].

However, the technique remains insufficient; metabolic control is partial and durability of the result is limited to less than one year in most cases. Our aim is to review the current knowledge on the role of LCT to treat CN patients, report two additional patients, and review animal experiments performed as preclinical studies.

LCT FOR CN DISEASE TYPE I

Lessons from the animal model

The Gunn rat model represents the rodent equivalent of CN disease and is characterized by a single mutation in the *ugt1A1* gene. In this model, many experimental protocols using free or encapsulated liver cells have been designed with syngeneic/congeneic or allogeneic transplantation procedures[21-32]. Table 1 summarizes representative experiments. The best results were obtained when a hepatic injury was caused before LCT to create a niche and a regenerative stimulus for engrafting cells. The explanation for why the injury was beneficial is Gunn rats global liver function is normal, except for bilirubin conjugation, and the lack of host hepatocyte impairment fails to provide to donor cells a proliferative advantage. The repopulation rate necessary to observe a metabolic efficacy ranges from 5% to 10%[33]. Significant lowering of serum bilirubin could be observed up to 12 mo while using congenic procedures[27].

On the clinical side

Reports of human LCT for CN disease have shown encouraging results. The first demonstration of the efficacy of the technique was provided by Fox *et al*[14]. In this case, 7.5×10^9 viable liver cells were infused in a 10-year-old patient and the effect was a significant decrease of bilirubin levels for up to 11 mo (Table 2). UGT1A1 enzyme activity was detected in the host liver and glucuronoconjugates were found in bile confirming

Table 2 Summary of clinical liver cell transplantation procedures for liver-based inborn errors of metabolism

Indication	*n*	Patient age	Cell amount (% liver cell mass)	Follow-up	References
Familial hypercholesterolemia	5	7-41 yr		Partial reduction of LDL (3/5 patients) Donor hepatocytes detected by ISH at 4 mo	69
	1	10 yr	7.5×10^9 (5%)	Decrease of bilirubin levels up to 11 mo Detection of UGT1A1 enzyme activity and of glucurono-conjugates in bile	14
CN disease type I	1	9 yr	7.5×10^9 (5%)	50%-65% reduction of bilirubin up to 3 mo Donor hepatocytes not detected by short tandem repeat analysis at 40 d	34
	2	18 mo/3 yr	ND	50%/30% reduction of serum bilirubin over 7 mo/ND follow-up Donor hepatocytes detected in one case by short tandem repeat analysis at 8 mo	33
Infantile refsum disease	1	4 yr	2×10^9	Donor Y-chromosomes detected by PCR at 7 d	20
Inherited coagulation factor Ⅶ deficiency	2	3 mo/2 yr	1.1×10^9/ 2.2×10^9 (4%/3%)	Decrease in the factor Ⅶ requirements	19
PFIC 2	2	ND	0.3×10^9	No improvement	33
Glycogen storage disease type I a	1	47 yr	2×10^9 (1%)	Fasting tolerance: up to 7 h Increase of glycemia Improvement of diet G6Pase activity detected	18
Urea cycle disease	1 (OTC)	5 yr	1×10^9	Improvement of ammonia levels Detection of enzyme activity	70
	1 (OTC)	0 d	10.5×10^9	Transient metabolic improvement between 20 and 31 d of life	71
	1 (OTC)	1 d	1.9×10^9	Improvement of ammonia levels Increased urea synthesis	72
	1 (OTC)	14 mo	2.4×10^9 (6%)	Improvement of psychomotor development and of ammonia levels Urea neo-synthesis	16
	1 (ASL)	3.5 yr	4.7×10^9 (9%)	Improvement of psychomotor development and of ammonia levels Donor hepatocytes detected by FISH at 12 mo and by enzyme activity at 8 mo	17

ASL: Arginino-succinate lyase; (F)ISH: (Fluorescent) in situ hybridization; LDL: Low density lipoproteins; ND: Not documented; OTC: Ornithine transcarbamylase; PCR: Polymerase chain reaction; PFIC: Progressive familial intrahepatic cholestasis.

Table 3 Presentation of LCT procedures in two Crigler-Najjar disease type I patients

	Patient 1	Patient 2
Age/Gender	9 yr/Female	1 yr/Female
Infusion procedure	Porth-a-cath in jejunal vein	Broviac in portal vein
Timing of infusions	18 infusions/5 mo	14 infusions/15 d
Donor cells	Fresh and cryopreserved from 3 donors	Fresh and cryopreserved from 1 donor
Cell amount	6.1 billion 0.16 billion/kg	2.6 billion 0.35 billion/kg
% Liver cell mass	4%	8%
Mean viability	80%	83%

the integration of functional, healthy hepatocytes. Dhawan *et al* reported two additional patients ages 18 mo and 3 years, in which the reduction of serum bilirubin reached up to 50% and 30%, respectively over a follow-up period up of 7 mo (Table 2). Donor hepatocyte engraftment was illustrated by short tandem repeat analysis at 8 mo follow-up. Ambrosino *et al* also described a decrease of bilirubin levels up to 3 mo post-LCT, whereas they did not detect donor cells by using a short tandem repeat assay at 40 d follow-up[34].

We performed LCT in two CN pediatric cases (Table 3, Figure 1). The first patient was a 9 year old girl in whom a port-a-cath was placed in the jejunal vein. She received 18 cell infusions from three different donors over a period of 5 mo for a total of 4% of her estimated liver cell mass. Mean cell viability was high (80%) and no adverse events were noticed during the procedure. Pre-transplant serum bilirubin values attained 17.5 ± 0.49 mg/dL (mean ± SD) and dropped after LCT to the lowest value of 11.4 mg/dL (mean ± SD: 13.6 ± 0.42 mg/dL, $P < 0.001$). After a period of 6 mo, bilirubin values increased suddenly without a concomitant event and the patient was scheduled for OLT. For the second patient, the protocol was revised in order to provide a higher amount of cells within a shorter infusion period. She was 1 year old at the time

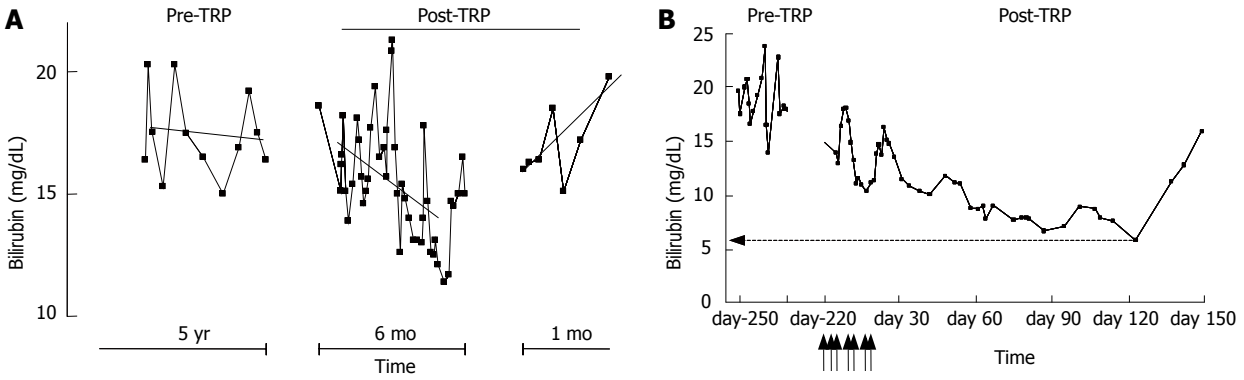

Figure 1 Evolution of serum bilirubin before and after LCT in two CN patients performed in our center. **A:** After fluctuating over a period of 5 yr, serum bilirubin of patient 1 decreased significantly to the lesser value of 11.4 mg/dL in 6 mo. Subsequently, increasing values were observed and the patient was listed for OLT. **B:** For patient 2, after cell infusions, the serum bilirubin dramatically decreased to the value of 6 mg/dL in 4 mo. At this time, concomitantly to an EBV infection, higher values were observed and the patient underwent OLT. Arrows indicate the timing of cell infusions. TRP: Transplantation.

of the procedure and received 14 infusions from one single donor over 15 d to reach a total of 8.6% of her estimated liver cell mass. Cells were infused *via* a broviac catheter surgically inserted *via* a colonic vein to the spleno-mesaraïc confluent. Cell viability (mean 83%) and clinical tolerance were optimal. With pre-LCT levels of 17.6 ± 3.5 mg/dL (mean ± SD), the serum bilirubin dramatically decreased to values of 13.3 ± 2.4 mg/dL (mean ± SD) with the lowest value at 6 mg/dL. Skin jaundice reduced rapidly and the daily phototherapy schedule was alleviated from 10 to 8 h without any influence on the bilirubin levels. After 4 mo of progressive decrease of serum bilirubin, the values increased suddenly following an intercurrent Epstein-Barr virus (EBV) infection. The child underwent OLT without complications related to the previous LCT. Both patients received a methylprednisolone bolus and tacrolimus the day before and for 12 d after LCT. Subsequently they were given tacrolimus as long-term monotherapy.

PERSPECTIVES

At present, LCT remains limited by incomplete and time-limited metabolic control, mainly due to unfavorable immunological cell interactions, impaired donor cell quality and poor repopulation rates. Whereas the immunogenicity of liver cells is quite different compared to whole liver[35], the same immunosuppression protocols are applied for LCT and OLT. Additional fundamental *in vivo* studies are necessary for the development of the optimal immunosuppression protocol. In that way, Wu *et al* recently compared the effects of tacrolimus, rapamycin and mycophenolate mofetil on the engraftment and proliferation of engrafted liver cells in a allogeneic setting[36]. They observed a deleterious effect of rapamycin on the proliferation of the transplanted cells. Serrano *et al* reported the lack of toxicity of tacrolimus and methylprednisolone on human hepatocytes *in vitro*[37]. Other experimental protocols were designed to reduce the immunological pressure occurring in LCT procedures. For example, Mashalova *et al* obtained similar engraftment levels with syngeneic or allogeneic hepatocytes after their transduction with adenoviral early region 3 genes, suggesting a protective effect against rejection[38]. This was related to the down-expression of *Fas* receptor at the cell surface leading to inhibition of Fas-mediated apoptosis. Protocols combining LCT with bone marrow transplantation with[39] or without[40] elimination of natural killer cells are being investigated. Liver cell encapsulation aiming to protect cells from the immune system has demonstrated promising results in Gunn rats[41-43]. The technique is reversible and allows delivery of the cells to extrahepatic sites that are easy to access for sampling. However, major remaining hurdles are the creation of an adequate 'intracapsular' microenvironment allowing long-term cell functionality and the restriction of this technique to an enzyme-delivery role. Host immunity can be modulated by co-transplantation of immunomodulatory cells, as developed by Le Blanc *et al,* using mesenchymal stem cells to control graft *versus* host disease in the bone marrow transplant setting[44,45]. These cells and others, as non-parenchymal cells[46] or liver-derived mesenchymal lineages[47,48], could provide permissive factors or a microenvironment allowing more favorable immunological cell interactions, although this has not been tested so far in LCT protocols. Study of inner mechanisms of cell rejection may also lead to improved clinical efficiency of LCT. For example, it has been shown recently that human hepatocytes exert a procoagulant activity depending on tissue factor expression[49], as previously demonstrated with pancreatic islet cells[50,51]. In this work, Stéphenne *et al* demonstrated the improvement of the procoagulant activity by incubating the cells with N-acetylcysteine, making this drug valuable for additional *in vivo* studies.

Enhancement of liver cell engraftment capacity is another challenge. Engraftment depends on liver cell quality and host liver environment. While LCT is highly dependent on banking of cryopreserved cells, this procedure has been demonstrated to deteriorate cell quality. Indeed, although cryopreserved/thawed hepatocytes have been shown to possess *in vivo* clonal

replicative potential identical to freshly isolated cells[52], their *in vivo* potential seems to be restricted in time[53-55] and their *in vitro* functionality remains lower than that of freshly isolated hepatocytes[56]. Furthermore, we recently demonstrated that, with the current protocols, cryopreservation/thawing of hepatocytes induces cell alteration and especially mitochondrial defects (complex 1 impairment)[57]. Intracellular ice formation remains the major factor affecting the quality of cells. Protection delivered by non-permeating cryoprotectants must be further analyzed in terms of cell death and mitochondrial functions. New perspectives, such as vitrification, to avoid the crystalline state, coupled or not with encapsulation, must be validated in the future while considering the problem of hepatocyte de-differentiation at long term that could occur in this type of configuration.

Actions on the liver microenvironment have been evaluated in a recent report using monocrotaline, which is an alkaloid showing toxicity against liver endothelial and Kupffer cells[58]. Authors reported an enhanced liver cell engraftment in a syngeneic background mainly related to endothelial cell damage. Comparable studies were performed on dipeptidyl peptidase $IV^{-/-}$F344 rats using doxorubicin, irinotecan, or vincristine[59]. In this study, Kim *et al* showed improved cell engraftment after doxorubicin treatment attributed to endothelial cell disruption. While interesting, these approaches will not be applicable in a clinical setting. Physical alteration of the liver architecture was studied by Dagher *et al* on nonhuman primates using partial portal vein ligation or embolization in an autologous LCT procedure[60]. The authors reported hepatic regeneration rates up to 10% obtained at short term (15 d) after embolization of the portal vein. Others have successfully used chemicals as vascular endothelial growth factor delivered *in situ*[61] or by peripheral route[62] to promote cell engraftment.

As stem cells were recently described to have a hepatocyte differentiation potential[63,64], these are currently considered with growing interest for liver cell therapy. The most potent candidates are mesenchymal stem cells isolated from various tissues, with predilection for bone marrow[65] and umbilical cord[66]. Liver progenitor cells[67] or mesenchymal-like cells[47,48] also deserve detailed attention. However, stem cells only display partial hepatocyte-like functionality[64,68] and further advance is necessary to consider such cell types for therapy.

CONCLUSION

While LCT seems currently efficient and safe to improve the quality of life of CN diseased patients for a medium period of time, the technique still requires development to be considered for longer term or curative purposes. Advances must be focused on the quality of cell preparations together with the management of immunological barriers hampering reliable cell engraftment. Furthermore, other research areas, such as gene or stem cell therapy, are currently encountering exciting expansion, and combined therapeutic approaches would be justified in the near future.

REFERENCES

1 **Bosma PJ**, Seppen J, Goldhoorn B, Bakker C, Oude Elferink RP, Chowdhury JR, Chowdhury NR, Jansen PL. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. *J Biol Chem* 1994; **269**: 17960-17964

2 **Kaufman SS**, Wood RP, Shaw BW Jr, Markin RS, Rosenthal P, Gridelli B, Vanderhoof JA. Orthotopic liver transplantation for type I Crigler-Najjar syndrome. *Hepatology* 1986; **6**: 1259-1262

3 **Sokal EM**, Silva ES, Hermans D, Reding R, de Ville de Goyet J, Buts JP, Otte JB. Orthotopic liver transplantation for Crigler-Najjar type I disease in six children. *Transplantation* 1995; **60**: 1095-1098

4 **Evans HM**, Kelly DA, McKiernan PJ, Hubscher S. Progressive histological damage in liver allografts following pediatric liver transplantation. *Hepatology* 2006; **43**: 1109-1117

5 **Heaton N**. Small-for-size liver syndrome after auxiliary and split liver transplantation: donor selection. *Liver Transpl* 2003; **9**: S26-S28

6 **Bellodi-Privato M**, Aubert D, Pichard V, Myara A, Trivin F, Ferry N. Successful gene therapy of the Gunn rat by in vivo neonatal hepatic gene transfer using murine oncoretroviral vectors. *Hepatology* 2005; **42**: 431-438

7 **van der Wegen P**, Louwen R, Imam AM, Buijs-Offerman RM, Sinaasappel M, Grosveld F, Scholte BJ. Successful treatment of UGT1A1 deficiency in a rat model of Crigler-Najjar disease by intravenous administration of a liver-specific lentiviral vector. *Mol Ther* 2006; **13**: 374-381

8 **Seppen J**, Bakker C, de Jong B, Kunne C, van den Oever K, Vandenberghe K, de Waart R, Twisk J, Bosma P. Adeno-associated virus vector serotypes mediate sustained correction of bilirubin UDP glucuronosyltransferase deficiency in rats. *Mol Ther* 2006; **13**: 1085-1092

9 **Seppen J**, van Til NP, van der Rijt R, Hiralall JK, Kunne C, Elferink RP. Immune response to lentiviral bilirubin UDP-glucuronosyltransferase gene transfer in fetal and neonatal rats. *Gene Ther* 2006; **13**: 672-677

10 **Seppen J**, Tada K, Ottenhoff R, Sengupta K, Chowdhury NR, Chowdhury JR, Bosma PJ, Oude Elferink RP. Transplantation of Gunn rats with autologous fibroblasts expressing bilirubin UDP-glucuronosyltransferase: correction of genetic deficiency and tumor formation. *Hum Gene Ther* 1997; **8**: 27-36

11 **Nguyen TH**, Birraux J, Wildhaber B, Myara A, Trivin F, Le Coultre C, Trono D, Chardot C. Ex vivo lentivirus transduction and immediate transplantation of uncultured hepatocytes for treating hyperbilirubinemic Gunn rat. *Transplantation* 2006; **82**: 794-803

12 **Galbraith RA**, Drummond GS, Kappas A. Suppression of bilirubin production in the Crigler-Najjar type I syndrome: studies with the heme oxygenase inhibitor tin-mesoporphyrin. *Pediatrics* 1992; **89**: 175-182

13 **Kren BT**, Parashar B, Bandyopadhyay P, Chowdhury NR, Chowdhury JR, Steer CJ. Correction of the UDP-glucuronosyltransferase gene defect in the gunn rat model of crigler-najjar syndrome type I with a chimeric oligonucleotide. *Proc Natl Acad Sci USA* 1999; **96**: 10349-10354

14 **Fox IJ**, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 1998; **338**: 1422-1426

15 **Najimi M**, Sokal E. Liver cell transplantation. *Minerva Pediatr* 2005; **57**: 243-257

16 **Stephenne 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**: 2058-2061
17 **Stephenne 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**: 1317-1323
18 **Muraca M**, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, Giron G, Burlina AB. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet* 2002; **359**: 317-318
19 **Dhawan A**, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, Arya R, Wade JJ, Verma A, Heaton ND, Rela M, Mieli-Vergani G. Hepatocyte transplantation for inherited factor VII deficiency. *Transplantation* 2004; **78**: 1812-1814
20 **Sokal EM**, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, Evrard V, Latinne D, Vincent MF, Moser A, Soriano HE. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* 2003; **76**: 735-738
21 **Cobourn CS**, Makowka L, Falk JA, Falk RE. Allogeneic intrasplenic hepatocyte transplantation in the Gunn rat using cyclosporine A immunosuppression. *Transplant Proc* 1987; **19**: 1002-1003
22 **Dixit V**, Darvasi R, Arthur M, Brezina M, Lewin K, Gitnick G. Restoration of liver function in Gunn rats without immunosuppression using transplanted microencapsulated hepatocytes. *Hepatology* 1990; **12**: 1342-1349
23 **te Velde AA**, Bosman DK, Oldenburg J, Sala M, Maas MA, Chamuleau RA. Three different hepatocyte transplantation techniques for enzyme deficiency disease and acute hepatic failure. *Artif Organs* 1992; **16**: 522-526
24 **Zhang H**, Miescher-Clemens E, Drugas G, Lee SM, Colombani P. Intrahepatic hepatocyte transplantation following subtotal hepatectomy in the recipient: a possible model in the treatment of hepatic enzyme deficiency. *J Pediatr Surg* 1992; **27**: 312-315; discussion 315-316
25 **Holzman MD**, Rozga J, Neuzil DF, Griffin D, Moscioni AD, Demetriou AA. Selective intraportal hepatocyte transplantation in analbuminemic and Gunn rats. *Transplantation* 1993; **55**: 1213-1219
26 **Albani AP**, Campanati L, Arosio E, Gatti S, Gridelli B, Orsenigo R, Grizzi F, Doglia M, Fassati LR, Galmarini D. Hepatocyte injection in Gunn rats' thymus and spleen. *Transplant Proc* 1994; **26**: 3443-3445
27 **Kokudo N**, Otsu I, Okazaki T, Takahashi S, Sanjo K, Adachi Y, Makino S, Nozawa M. Long-term effects of intrasplenically transplanted adult hepatocytes and fetal liver in hyperbilirubinemic Gunn rats. *Transpl Int* 1995; **8**: 262-267
28 **Ilan Y**, Roy-Chowdhury N, Prakash R, Jona V, Attavar P, Guha C, Tada K, Roy-Chowdhury J. Massive repopulation of rat liver by transplantation of hepatocytes into specific lobes of the liver and ligation of portal vein branches to other lobes. *Transplantation* 1997; **64**: 8-13
29 **Guha C**, Parashar B, Deb NJ, Garg M, Gorla GR, Singh A, Roy-Chowdhury N, Vikram B, Roy-Chowdhury J. Normal hepatocytes correct serum bilirubin after repopulation of Gunn rat liver subjected to irradiation/partial resection. *Hepatology* 2002; **36**: 354-362
30 **Kim BH**, Han YS, Dong SH, Kim HJ, Chang YW, Lee JI, Chang R. Temporary amelioration of bilirubin conjugation defect in Gunn rats by transplanting conditionally immortalized hepatocytes. *J Gastroenterol Hepatol* 2002; **17**: 690-696
31 **Takahashi M**, Deb NJ, Kawashita Y, Lee SW, Furgueil J, Okuyama T, Roy-Chowdhury N, Vikram B, Roy-Chowdhury J, Guha C. A novel strategy for in vivo expansion of transplanted hepatocytes using preparative hepatic irradiation and FasL-induced hepatocellular apoptosis. *Gene Ther* 2003; **10**: 304-313
32 **Cubero FJ**, Maganto P, Mula N, Ortiz A, Barrutia MG, Codesal FJ, Arahuetes RM. Hepatic proliferation in Gunn rats transplanted with hepatocytes: effect of retrorsine and tri-iodothyronine. *Cell Prolif* 2005; **38**: 137-146
33 **Dhawan A**, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. *J Inherit Metab Dis* 2006; **29**: 431-435
34 **Ambrosino G**, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, Basso S, Carraro P, Valente ML, D'Amico D, Zancan L, D'Antiga L. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. *Cell Transplant* 2005; **14**: 151-157
35 **Crispe IN**, Giannandrea M, Klein I, John B, Sampson B, Wuensch S. Cellular and molecular mechanisms of liver tolerance. *Immunol Rev* 2006; **213**: 101-118
36 **Wu YM**, Joseph B, Gupta S. Immunosuppression using the mTOR inhibition mechanism affects replacement of rat liver with transplanted cells. *Hepatology* 2006; **44**: 410-419
37 **Serrano T**, Mitry RR, Terry C, Lehec SC, Dhawan A, Hughes RD. The effects of immunosuppressive agents on the function of human hepatocytes in vitro. *Cell Transplant* 2006; **15**: 777-783
38 **Mashalova EV**, Guha C, Roy-Chowdhury N, Liu L, Fox IJ, Roy-Chowdhury J, Horwitz MS. Prevention of hepatocyte allograft rejection in rats by transferring adenoviral early region 3 genes into donor cells. *Hepatology* 2007; **45**: 755-766
39 **Wesolowska A**, Olszewski WL, Durlik M. Transplantation of hepatocytes: elimination of recipient natural killer cells with irradiation and bone marrow reconstitution prevent early graft dysfunction. *Transplant Proc* 2003; **35**: 2358-2360
40 **Yoshida N**, Kawahara T, Futagawa S. Induction of donor-specific tolerance to allogeneic hepatocytes by allogeneic bone marrow transplantation. *Hepatol Res* 2003; **26**: 148-153
41 **Dixit V**, Darvasi R, Arthur M, Lewin K, Gitnick G. Cryopreserved microencapsulated hepatocytes--transplantation studies in Gunn rats. *Transplantation* 1993; **55**: 616-622
42 **Gomez N**, Balladur P, Calmus Y, Baudrimont M, Honiger J, Delelo R, Myara A, Crema E, Trivin F, Capeau J, Nordlinger B. Evidence for survival and metabolic activity of encapsulated xenogeneic hepatocytes transplanted without immunosuppression in Gunn rats. *Transplantation* 1997; **63**: 1718-1723
43 **Liu ZC**, Chang TM. Coencapsulation of hepatocytes and bone marrow stem cells: in vitro conversion of ammonia and in vivo lowering of bilirubin in hyperbilirubemia Gunn rats. *Int J Artif Organs* 2003; **26**: 491-497
44 **Le Blanc K**. Immunomodulatory effects of fetal and adult mesenchymal stem cells. *Cytotherapy* 2003; **5**: 485-489
45 **Le Blanc K**, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, Ringden O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; **363**: 1439-1441
46 **Ding XM**, Xue WJ, Ji ZZ, Tian PX. Infusion of donor hepatic non-parenchymal cells prolongs survival of skin allografts in mice: role of microchimerism and IL-4. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 34-37
47 **Herrera MB**, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 2006; **24**: 2840-2850
48 **Najimi M**, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, Sokal EM. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? *Cell Transplant* 2007; **16**: 717-728
49 **Stephenne X**, Vosters O, Najimi M, Beuneu C, Dung KN, Wijns W, Goldman M, Sokal EM. Tissue factor-dependent procoagulant activity of isolated human hepatocytes: relevance to liver cell transplantation. *Liver Transpl* 2007; **13**: 599-606
50 **Beuneu C**, Vosters O, Movahedi B, Remmelink M, Salmon

I, Pipeleers D, Pradier O, Goldman M, Verhasselt V. Human pancreatic duct cells exert tissue factor-dependent procoagulant activity: relevance to islet transplantation. *Diabetes* 2004; **53**: 1407-1411

51 **Beuneu C**, Vosters O, Ling Z, Pipeleers D, Pradier O, Goldman M, Verhasselt V. N-Acetylcysteine derivative inhibits procoagulant activity of human islet cells. *Diabetologia* 2007; **50**: 343-347

52 **Jamal HZ**, Weglarz TC, Sandgren EP. Cryopreserved mouse hepatocytes retain regenerative capacity in vivo. *Gastroenterology* 2000; **118**: 390-394

53 **David P**, Alexandre E, Chenard-Neu MP, Audet M, Wolf P, Jaeck D, Azimzadeh A, Richert L. Engraftment and function of freshly isolated and cryopreserved Sprague Dawley rat hepatocytes after intrasplenic transplantation in analbuminemic rats. *Transplant Proc* 2000; **32**: 2796-2797

54 **David P**, Alexandre E, Audet M, Chenard-Neu MP, Wolf P, Jaeck D, Azimzadeh A, Richert L. Engraftment and albumin production of intrasplenically transplanted rat hepatocytes (Sprague-Dawley), freshly isolated versus cryopreserved, into Nagase analbuminemic rats (NAR). *Cell Transplant* 2001; **10**: 67-80

55 **Fuller BJ**, Lewin J, Sage L. Ultrastructural assessment of cryopreserved hepatocytes after prolonged ectopic transplantation. *Transplantation* 1983; **35**: 15-18

56 **Loven AD**, Olsen AK, Friis C, Andersen B. Phase I and II metabolism and carbohydrate metabolism in cultured cryopreserved porcine hepatocytes. *Chem Biol Interact* 2005; **155**: 21-30

57 **Stephenne X**, Najimi M, Ngoc DK, Smets F, Hue L, Guigas B, Sokal EM. Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex 1. *Cell Transplant* 2007; **16**: 409-419

58 **Joseph B**, Kumaran V, Berishvili E, Bhargava KK, Palestro CJ, Gupta S. Monocrotaline promotes transplanted cell engraftment and advances liver repopulation in rats via liver conditioning. *Hepatology* 2006; **44**: 1411-1420

59 **Kim KS**, Joseph B, Inada M, Gupta S. Regulation of hepatocyte engraftment and proliferation after cytotoxic drug-induced perturbation of the rat liver. *Transplantation* 2005; **80**: 653-659

60 **Dagher I**, Boudechiche L, Branger J, Coulomb-Lhermine A, Parouchev A, Sentilhes L, Lin T, Groyer-Picard MT, Vons C, Hadchouel M, Pariente D, Andreoletti M, Franco D, Weber A. Efficient hepatocyte engraftment in a nonhuman primate model after partial portal vein embolization. *Transplantation* 2006; **82**: 1067-1073

61 **Kedem A**, Perets A, Gamlieli-Bonshtein I, Dvir-Ginzberg M, Mizrahi S, Cohen S. Vascular endothelial growth factor-releasing scaffolds enhance vascularization and engraftment of hepatocytes transplanted on liver lobes. *Tissue Eng* 2005; **11**: 715-722

62 **Shani-Peretz H**, Tsiperson V, Shoshani G, Veitzman E, Neufeld G, Baruch Y. HVEGF165 increases survival of transplanted hepatocytes within portal radicles: suggested mechanism for early cell engraftment. *Cell Transplant* 2005; **14**: 49-57

63 **Nussler A**, Konig S, Ott M, Sokal E, Christ B, Thasler W, Brulport M, Gabelein G, Schormann W, Schulze M, Ellis E, Kraemer M, Nocken F, Fleig W, Manns M, Strom SC, Hengstler JG. Present status and perspectives of cell-based therapies for liver diseases. *J Hepatol* 2006; **45**: 144-159

64 **Lysy PA**, Campard D, Smets F, Najimi M, Sokal EM. Stem cells for liver tissue repair: current knowledge and perspectives. *World J Gastroenterol* 2008; **14**: 864-875

65 **Lysy PA**, Campard D, Smets F, Malaise J, Mourad M, Najimi M, Sokal EM. Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells. *Cell Prolif* 2008; **41**: 36-58

66 **Campard D**, Lysy PA, Najimi M, Sokal EM. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. *Gastroenterology* 2008; **134**: 833-848

67 **Fiegel HC**, Lange C, Kneser U, Lambrecht W, Zander AR, Rogiers X, Kluth D. Fetal and adult liver stem cells for liver regeneration and tissue engineering. *J Cell Mol Med* 2006; **10**: 577-587

68 **Hengstler JG**, Brulport M, Schormann W, Bauer A, Hermes M, Nussler AK, Fandrich F, Ruhnke M, Ungefroren H, Griffin L, Bockamp E, Oesch F, von Mach MA. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. *Expert Opin Drug Metab Toxicol* 2005; **1**: 61-74

69 **Grossman M**, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ 3rd, Stein EA, Lupien PJ, Brewer HB Jr, Raper SE. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. *Nat Med* 1995; **1**: 1148-1154

70 **Strom SC**, Fisher RA, Rubinstein WS, Barranger JA, Towbin RB, Charron M, Mieles L, Pisarov LA, Dorko K, Thompson MT, Reyes J. Transplantation of human hepatocytes. *Transplant Proc* 1997; **29**: 2103-2106

71 **Horslen SP**, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, Fox IJ. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics* 2003; **111**: 1262-1267

72 **Mitry RR**, Dhawan A, Hughes RD, Bansal S, Lehec S, Terry C, Heaton ND-, Karani JB, Mieli-Vergani G, Rela M. One liver, three recipients: segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. *Transplantation* 2004; **77**: 1614-1616

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