

Human Autologous Liver Cell Transplantation for the Treatment of Cirrhosis

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Citation

A Schwarz, T Lindl, C H hneke, M Stange, W Pieken. *Human Autologous Liver Cell Transplantation for the Treatment of Cirrhosis*. The Internet Journal of Gastroenterology. 2009 Volume 10 Number 1.

Abstract

We report here the application of autologous liver cell transplantation on a polymer scaffold for the therapy of human liver cirrhosis. A liver tissue sample and a pancreas biopsy were harvested from cirrhotic patients. Vital liver and pancreas cells were isolated from these tissue samples, seeded onto a poly-L-lactic acid matrix and re-implanted into the mesentery of the same patient. The autologous matrix assisted co-transplantation of liver cells together with a small amount of pancreatic cells was applied to 57 individual treatments of liver cirrhosis. The average survival rate following one year after transplantation was 75 % for all patients. For those with MELD ≤ 10 , the one year survival was 91 %. The average MELD score stayed constant for 37 patients for whom 12 or 24 months follow up data was available. This is in contrast to the literature, which reports a significant degradation in MELD score for patients treated conventionally. For a majority of patients, the liver related blood values remained stable or improved 12 months post treatment, except for the gamma GT value, which in most cases did not improve. The majority of patients also reported improved quality of life one year post treatment. The autologous matrix-seeded transplantation of liver cells warrants further controlled clinical study for the stabilization and possibly bridging to orthotopic liver transplantation of cirrhotic patients.

INTRODUCTION

Currently, the only treatment available for end stage liver disease is orthotopic liver transplantation. This option is limited by the scarce supply of organs and is complicated by requiring chronic immunosuppression to avoid graft rejection. Extensive research into the transplantation of liver cells or hepatocytes has been pursued with the aim to find an alternative treatment method for end stage liver disease and acute liver failure (ALF) [1,2],[3]. Several attempts at human hepatocyte transplantations have been reported for treatment of ALF and liver based metabolic diseases with varying success [4]. In these studies the main route of administration of the cells was either injection into the spleen or direct injection into the portal vein.

Over the past two decades the tissue engineering approach has emerged as a new method to resurrect or augment organ function [5]. For augmentation of liver function, the matrix assisted transplantation of hepatocytes has been developed as a method that provides an optimal environment for the proliferation of the transplanted cells [6]. In an early experiment the concept of seeding cells on a polymer matrix and subsequent transplantation of the cell-matrix construct was successfully demonstrated in rats [7]. Biodegradable

polymer surfaces [8] and in particular porous poly-L-lactic acid (PLLA) membranes were established as suitable media to support hepatocyte adhesion and function[9],[10]. In order to exert their function in vivo, implants need to be positioned such that they are readily vascularized and can participate in metabolic exchange. The mesentery tissue was shown to provide a suitable environment for hepatocyte matrix implants in Gunn rats [11]. In this animal model, implant biopsies taken after 99 days showed neovascularization and hepatocyte function. The study also proved that permanent Bilirubin conjugation could be restored in protein-deficient Gunn rats after receiving transplants from congenic rats. Not only rat cells but also human hepatocyte samples on polymer matrices implanted into athymic rats [12] showed at least transient albumin production after 7 days.

Transplanted rat hepatocytes, stimulated by a portocaval shunt, in Lewis rats were found to be viable and do proliferate even after one year post implantation [13].

The requirement for stimulation of the hepatocytes implanted into the mesentery can also be met by co-transplantation of a small amount of islets of Langerhans [14,15]. This stimulatory effect of islet cells on transplanted

hepatocytes was also observed in athymic mice [16] In rats the optimal ratio of islets for stimulation of polymer implants was found to be 40 islets of Langerhans per 1 million hepatocytes. [17]

In a recent review, Fisher and Strom have summarized the human hepatocyte transplantations [1]. They include autologous hepatocyte transplants in cirrhotic patients by injection into the spleen in a total of 10 patients.

Taken together, these findings prompted us to adapt and apply the autologous liver cell transplantation on highly porous PLLA matrices to the individual therapy of chronic liver disease. Here we report the results from 57 transplantations.

MATERIALS AND METHODS

Patients with liver cirrhosis were screened for general fitness, mental fitness, the progression of liver disease, the etiology of the liver disease, and other disease conditions. Only patients with an established cirrhosis of non-viral background were admitted to the treatment.

Autologous liver tissue is obtained by a median laparotomy of the upper abdomen with an incision of 12 cm under general anesthesia and standard antibiotic prophylaxis with cefazolin and metronidazol. The left lobe of the liver is mobilized. A partial resection of segment 3 of the liver is performed. Control of bleed is managed by single U-shaped stitch using Vicryl 2x0 and the use of an Argon laser for surface coagulation.

Autologous pancreatic tissue is obtained by opening the omental bursa to expose the pancreas. In the middle of the pancreas, two resorbable PDS 5x0 sutures are placed and a small lobe of the pancreas is excised.

The resected tissues are collected in sterile cold Custodiol® transplant solution, and kept cold with crushed ice during transport to the tissue laboratory for processing. A 50 ml tube of patient serum is also collected at the same time as the autologous tissue specimens.

ISOLATION OF LIVER SINGLE CELL SUSPENSION:

The liver cells are isolated essentially as described in the literature [18]. Prior to digestion the liver segment was perfused with EGTA-solution with up to eight needle injection points into blood vessels for up to 75 min, until the color of the liver segment changed from dark red to a consistent grey color.

The liver cell numbers isolated are determined with a haemocytometer (Neubauer Improved chamber) after staining the cell suspension with 0.2 % Trypan blue solution for two min. Also, microscopic observation for intact cell membranes of the viable cells under phase contrast microscopy is performed simultaneously. The viability of the liver cells was within the range of approximately 70% to 80%.

ISOLATION OF PANCREATIC SINGLE CELL SUSPENSION:

An approximately pea-sized pancreatic tissue is cut with sterile scalpels in a petri-dish and covered with 10 ml enzyme digestion solution (10 U/ml). The cut pieces are then incubated at 37°C with 5% CO₂ for 20 min. After the incubation period, the disintegrated pancreatic islet cells are passed through a nylon cell sieve (100 µm mesh size) with the aid of a sterile cell scraper (Fa. Sarstedt) and additional digestion solution is added to a final volume of 40 ml into a centrifuge tube (50 ml size). This filtrate is centrifuged at 100 x g for 5 min and the supernatant removed, with the cell pellet resuspended in warm Williams E medium with 5 % (vol.) of autologous serum. This wash procedure is repeated twice and the last cell pellet then resuspended in 2.1 ml of Williams E medium, completed with autologous serum. An aliquot (0.1 ml) is taken for cell number enumeration and viability check following exactly the liver cell protocol.

SEEDING OF THE SINGLE CELL SUSPENSION ON PLLA MATRICES:

The 2.0 ml of pancreatic islet cell solution and the 18 ml of liver cell solution are mixed and re-suspended into a 20 ml cell solution for preparation of the matrices (HeparAutoCell™ PLLA matrices, procured from HumanAutoCell GmbH).[19]

In each of the 20 wells of the multi-well plate, a sterile collagen coated matrix is placed, and 1 ml of the cell solution (the matrix cannot absorb more volume) is pipetted onto the top of each matrix piece, drop by drop, so that the suspension can be absorbed into the matrix. The loaded matrices are then incubated at 37°C, with 5% CO₂ and 95% relative humidity for one hour, to enable the cells to adhere. After one hour another 1 ml of medium, completed with autologous serum, is added to each well. The matrices are then incubated at 37°C, with 5% CO₂ and 95% relative humidity for a minimum of 36 hrs in order to let viable cells attach firmly onto the matrix surface. Typically 2.2 million cells are seeded per matrix.

The loaded matrices are transferred back to the operating site in 50 ml tubes, pre-filled with 20 ml warm (37 °C) Williams E medium, completed with autologous serum.

Two to three days after liver resection a second operation is performed at the hospital to transfer the liver cells to the mesentery of the patient's small intestine. For this purpose the previous abdominal opening is used. 20 tissue chips are inserted into the mesentery of the small intestine. Each is 2 cm in diameter and 4 mm thick, and loaded with liver cells. For this purpose, incisions about 2.5 cm long are made at ten different places in the mesothelium of the mesentery. Pockets are prepared in the layer of mesentery between the mesothelium and the fatty tissue. Two tissue chips are inserted in each pocket.

RESULTS

We report here on 57 Caucasian patients that received the matrix-seeded liver cell transplantation. The patients were between 20.8 and 79.4 years of age, with an average age of 59.7 years, and a median age of 61.5 years. The group of patients was comprised of 21 females (average age 69.9), and 36 males (average age 59.6). Of these, 43 suffered from an alcohol abuse related cirrhosis, 9 from cirrhosis of unknown origin, one from cirrhosis of toxic origin, one suffering an α 1-antitrypsin deficiency, one suffering from cirrhosis with a history of HBV infection and two with a history of HAV infection (no acute infection). At the entry into the treatment patients displayed a MELD score [20],[21] of between 6 and 21, with the average MELD score of 12 and a standard deviation of 4. MELD scores were calculated by using the UNOS method and online calculation tool [22].

Given the autologous nature of the procedure, the number of isolated cells per patient varied significantly, as did the physical condition of the resected liver tissue. The patients received between 1.7 and 171.7 million autologous liver and pancreatic cells during the transplantation, with an average of 30.0 million cells and a standard deviation of \pm 32.6. The amount of pancreatic cells varied from 0.02% of the total implanted cells to 17.3 % of the total implanted cells, with the average being 1.7 % and a standard deviation of 3.64 %.

Of the 57 patients, 43 (75 %) survived longer than one year post liver cell transplantation and 14 (25 %) died within 12 months post liver cell transplantation.

Figure 1

Table 1: Survival in dependence of MELD pre-treatment

Survival	MELD < 10	MELD 11 to 15	MELD 16 to 20	MELD > 20
TOTAL Patients	25	24	5	3
Survival after 3 months	25	21	2	1
Survival after 6 months	25	20	2	1
Survival after 9 months	25	19	2	1
Survival after 1 year	24	17	2	0

Recently, the risk of a surgical procedure for cirrhotic patients has been evaluated and correlated to the pre-procedure MELD score[23]. The study reports that patients undergoing major surgery of any kind suffer an increased mortality risk particularly within 90 days post surgery. This risk was reported to increase linearly with MELD above a MELD score of 8. This is consistent with the mortality rate observed in the group of patients with MELD higher than 10 in the present study (Table 1). However, unlike the risk reported for surgical procedures, we observed a 100 % 90 day survival rate for the group of MELD up to 10. This survival rate is also an improvement over the survival observed for similar patients in a broad review of prognostic indicators for survival in cirrhosis [24].

The MELD score has been specifically correlated to prediction of mortality in patients with alcoholic hepatitis [25]. Table 2 shows the MELD score development for those 37 patients for which a MELD score was recorded after 12 and/or 24 months post transplantation. The average and median MELD score for that group was 11 (+/- 3) pre-cell transplantation and remained constant at 12 and 24 months post cell transplantation. A total of two patients (5%) showed an increase of MELD above 5 within the 2 years, the majority of 33 patients (90%) showed a stable MELD within +/- 5 points of the pre-transplantation value, and two (5%) patient showed an improvement of MELD of greater than 5 points. This compares favorably to a study published by Saab et al (2006), [26] in which out of 429 patients listed for transplantation and treated conventionally 25% experienced a degradation of 5 MELD points or more after a mean follow up of 2.15 years (+/- 1.49 years). Furthermore, another study that observed the disease progression of listed patients reported a mortality rate of 10 % within the first year of observation of the patients with MELD < 10.[27]

Figure 2

Table 2: MELD score development

Patient	MELD Score prior to surgery	MELD 12 months	MELD 24 months	Gender	Age at entry	Etiology	vital Cell number implanted	% pancreas
01	10	7	10	F	63.61	Alc	8.60	17.33%
03	8	7	7	F	54.21	Alc	14.50	
07	10	10		M	33.82	Alc	22.20	11.99%
09	21	15		M	48.90	Alc	2.70	1.81%
10	12		19	F	74.56	Alc	26.30	3.48%
12	8	8		M	61.51	Alc	32.00	1.60%
13	6	6		F	71.76	ukn	9.80	1.63%
17	14	16	16	M	67.58	HAV	24.40	0.12%
18	6		7	F	56.33	Alc	16.80	0.15%
19	9	11	8	M	61.27	HAV	6.30	0.48%
20	13		15	M	66.20	ukn	38.70	0.17%
24	15		17	M	54.62	Alc	25.30	2.13%
25	14	15	9	M	71.61	Alc	6.90	0.36%
26	16		17	F	56.31	Alc	65.42	0.14%
27	10	8	6	M	67.70	Alc	39.24	0.16%
29	10		13	M	53.42	Alc	35.70	0.10%
32	7	7	9	M	46.21	Alc	22.00	0.04%
33	13	13	12	M	67.36	Alc	25.20	0.03%
34	10	10	14	M	65.47	Alc	22.45	0.07%
35	13		12	M	60.65	Alc	20.80	0.04%
36	11	10	10	F	68.82	ukn	32.90	0.45%
37	12	9	8	F	62.07	Alc	12.00	0.19%
38	10		8	M	66.27	Alc	11.20	0.20%
40	6	6	8	F	66.83	ukn	38.10	0.17%
41	17	8	8	M	50.11	Alc	9.50	0.20%
42	8	7	11	F	69.46	ukn	11.10	0.22%
43	10	9	7	F	46.52	Alc	50.00	0.02%
44	12	9		M	20.81	tox	1.70	7.18%
45	13	12		M	64.81	Alc	31.40	0.12%
46	7	9		F	66.73	ukn	16.60	0.18%
47	8	10		M	63.19	Alc	26.70	0.12%
48	10	15		M	79.39	ukn	11.20	0.32%
49	11	18		F	57.68	Alc	2.80	3.17%
50	8	7		F	57.73	Alc	49.90	0.08%
51	15	15		M	58.28	Alc	45.50	1.20%
52	13	9		M	58.31	Alc	17.10	0.20%
54	6	9		F	39.24	Alc	33.50	0.25%
Average	11	10	11		59.9		29.8	1.56%
Min	6	6	6		20.8		1.7	0.02%
Max	21	18	19		79.4		65.4	17.3%
Stdev	3	3	4		11.5		15.2	3.57%

The liver relevant blood parameters were monitored post treatment and were compared to the values at entry into treatment (Table 3). After 12 months post treatment, a majority of reporting patients experienced stable or improved values in transaminase (GOT, GPT), in liver synthesis parameters (CHE, Bilirubin, Albumin, Creatinine), and in blood coagulation (INR). In contrast, no improvement was observed by 81 % of the patients in gamma glutamyl transferase levels (GGT), a value indicative of biliary function. While no statistical analysis was possible for the individual treatments reported here, this may point to the function of the implanted hepatocytes, which are not expected to improve biliary function.

Figure 3

Table 3: Development of Blood Values 12 Months Post Treatment

Column1	Number of Patients Reporting	Number Stable or Improved	Number Degraded	% Stable or Improved
GOT	33	24	9	73%
GPT	30	19	11	63%
GGT	32	6	26	19%
CHE	25	13	12	52%
Bilirubin	34	19	15	56%
INR	35	27	8	77%
Albumin	21	11	10	52%
Creatinine	41	21	20	51%

For a subset of patients, the quality of life was compared 12 months post treatment and pre-treatment. The assessment of quality of life was performed with the Chronic Liver Disease Questionnaire (CLDQ) [28]. Of 24 reporting patients, 12 (50 %) scored a greater than 5 % improvement in quality of life, 8 (33%) reported a stable quality of life within +/- 5 %, and 4 (17%) reported a decrease in quality of life.

DISCUSSION

The experimental application of autologous matrix-seeded co-transplantation of liver cells with a small amount of pancreatic cells was demonstrated as a reliable procedure on 57 patients. The correlation between MELD score prior to treatment and survival time up to one year post transplantation shows that the procedure is safe and suggests that it is beneficial for patients with a MELD score below 10. Consistent with the observation of surgical risk in cirrhotic patients by others, [23] the risk of this procedure increased for the patient group with MELD above 10 and the procedure cannot be recommended for that group.

Remarkably, for those patients for which MELD was recorded 1 and 2 years post transplantation, an average stabilization of liver disease was observed, compared to a significant disease progression and 10 % mortality reported in comparable patients treated conventionally. The observed improvements in blood values and quality of life support the benefit of this treatment for compensated liver disease patients. This suggests that the autologous matrix-seeded transplantation of liver cells warrants further controlled clinical study for the stabilization and possibly bridging to orthotopic liver transplantation of cirrhotic patients.

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