Human Autologous Liver Cell Transplantation for the Treatment of Cirrhosis

A Schwarz, T Lindl, C Höhneke, M Stange, W Pieken

Citation

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 congeneric 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 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 be 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 α1-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 ±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 3.6%. 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.

Table 1: Survival in dependence of MELD pre-treatment

<table>
<thead>
<tr>
<th>Survival after</th>
<th>MELD &lt; 10</th>
<th>MELD 11 to 15</th>
<th>MELD 16 to 20</th>
<th>MELD &gt; 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL Patients</td>
<td>25</td>
<td>24</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Survival after 3 months</td>
<td>25</td>
<td>21</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Survival after 6 months</td>
<td>25</td>
<td>20</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Survival after 9 months</td>
<td>25</td>
<td>19</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Survival after 1 year</td>
<td>24</td>
<td>17</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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]
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.

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.

**References**

Human Autologous Liver Cell Transplantation for the Treatment of Cirrhosis


Author Information

A. Schwarz
Kreisklinik Biberach

T. Lindl
Institut für Angewandte Zellkultur

C. Höhneke

M. Stange
HAC Biomed GmbH

W. Pieken
HAC Biomed GmbH