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Chapter 5

Transplantation of autologous hepatocytes in spleen, pancreas, mesentery, stomach and small bowel wall in pigs

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ABSTRACT

Transplantation of isolated hepatocytes may provide a means for substitution of liver function. The aim of this study was to examine various implantation sites for hepatocyte transplantation in a large animal model, i.e. in the pig. The left lateral liver segments of pigs (n=9) were resected and perfused with collagenase. Isolated hepatocytes were injected into the parenchyma of the spleen, in the pancreas and mesentery and in the subserosa of stomach, jejunum and ileum. One animal served as control. At 1-3 months post-implantation, HIDA-Tc$^{99}$ was administered and total body scanning (TBSc) was performed (n=5). The spleen, pancreas, mesentery, stomach, small bowel wall and a piece of the remnant liver were excised for organ scanning (OSc). Finally, the implantation sites were excised and placed in a gamma counter for direct tissue counting, after which histopathological examination took place.

During TBSc, radioactivity could only be detected in the remnant liver. OSc showed activity in 2/8 pigs at the implantation sites in mesentery and small bowel wall. Radioactivity at these sites (direct tissue counting, DTC) was 10.4% and 42.0%, respectively, of radioactivity of the respective samples of the remnant liver. Radioactivity at the remaining injection sites in these animals, as well as in the other animals, showed only slightly or not elevated radioactivity when compared to control (sites injected with saline). Histological examination after 1-3 months showed clusters of morphologically normal hepatocytes at the implantation sites in stomach, bowel wall and mesentery in all animals. Only occasionally, hepatocytes were found in the pancreas in 2/8 animals. No hepatocytes could be detected in any of the implantation sites in the spleen.

These data demonstrate that in pigs, the mesentery and small bowel wall provide more favorable sites for transplantation of hepatocytes, compared to the pancreas or the spleen.

INTRODUCTION

Transplantation of the whole liver is currently the only well-established treatment of a spectrum of life-threatening liver diseases, including acute liver failure, hepatic cirrhosis, and several inherited metabolic diseases that result from an absence or deficiency of hepatocyte-derived gene products(7). Due to the shortage of donor organs and the costs of orthopic liver transplantation, several bioartificial liver devices are currently under investigation in multicentric trials. Hopefully they will prove to be effective in supporting patients with liver failure that are on the high urgency waiting list for liver transplantation. In inherited metabolic diseases of the liver, transplantation of hepatocytes obtained from an allogeneic donor liver could provide specific liver functions. Furthermore, transplantation of isolated, autologous hepatocytes may provide a means for hepatocyte-directed ex vivo gene therapy(4).

Much work has been done concerning hepatocyte transplantation in rodents, with intraportal and intrasplenic routes producing the best engraftment, function and cell survival(2). The optimal site and method for implantation of isolated hepatocytes in large animals still remains to be determined, however important in view of clinical application of hepatocyte transplantation. The aim of this study was to examine intraabdominal,
ectopic implantation sites for hepatocyte transplantation in a large animal model, i.e. in the pig.

MATERIALS AND METHODS

Animals
9 adult white female pigs were used, weighing between 40 and 44 kg. All procedures were approved by the institutional guidelines of the Animal Ethical Committee of the University of Amsterdam.

After fasting overnight, induction of anesthesia was achieved with intramuscular administration of ketamine (10mg/kg; Nimatec®, Eurovet, Bladel, the Netherlands), azaperon (2 mg/kg; Stresnil®, Janssen Pharmaceutica, Tilburg, The Netherlands) and atropine (0.02 mg/kg). After inhalation of a mixture of O₂:NO₂ (2:3) and isoflurane (0.4-1%, Abbott Laboratories Ltd., Queensborough, UK), pigs were endotracheally intubated and ventilated with a mixture of O₂ and air. Anesthesia was maintained by intravenous administration (0.5 ml/kg/hour) of a mixture of sufentanilcitrate (20mg/l, Janssen-Cilag, Tilburg, the Netherlands) and ketamine (20g/l). Muscle relaxation was obtained by intravenous administration (2ml/h) of pancuronium bromide (2 mg/ml, Organon Teknika B.V., Boxtel, the Netherlands).

Partial liver resection and hepatocyte isolation
A laparotomy was performed using a small upper abdominal midline incision. The portal vein and hepatic artery were dissected free in the hepatoduodenal ligament. The left lateral portal vein was selectively cannulated and flushed with saline. The discolored liver segments were resected using electrocauterity and vessel ligation and were immediately processed for hepatocyte isolation.

Hepatocytes were isolated according to a modified method as described by Seglen(6), by perfusing the portal vein of the excised livers segments with oxygenated Ca²⁺-free solution (37 Celsius) and a digestion buffer that contained 0.00625% (w/v) Liberase RH (Roche, Almere, The Netherlands). The capsule of the liver was opened and the digested parenchyma was collected on ice after filtration through surgical gauze. The cell suspension was diluted with ice-cold Hanks’ buffer solution and washed 3 times through centrifugation at 4°C and 50g for 3 min followed by resuspension in Hanks’ buffer solution to obtain the required concentration. The whole procedure until implantation took 2.5 – 3 hours, during which the animal was kept under anesthesia.

Grafting of hepatocytes
The hepatocyte suspension containing 40x 10⁶ cells/ml was slowly injected with a 25-gauge needle into the spleen (1.5 ml, while clamping of the vascular pedicle) and pancreas, mesentery, stomach and proximal and distal small bowel wall (0.25 – 0.5 ml). In the control animal, the same implantation sites were injected with saline. All implantation sites were marked with an inabsorbable suture. Postoperative pain was treated with Temgesic 0.005 – 0.01 mg/kg as clinically indicated.
**Graft assessment**

At 1-3 months post-implantation the animals were anaesthetized once more, and HIDA-Tc\textsuperscript{99} (200 MBq activity, in 20 ml) was administered intravenously, either during temporary clamping of the hepatoduodenal ligament (Pringle maneuver, n=4) or followed by total body scanning (n=5), after which the animals were sacrificed. The spleen, the pancreas, and the injected regions of mesentery, stomach and small bowel wall were subsequently excised and placed under the gammacamera for organ scanning (Osc). Finally, the implantation sites were excised, immersed in 4% formaldehyde, and placed in a gammacounter for direct tissue counting (DTC), after which histopathological examination took place of hematoxylin and eosin stained sections using brightfield microscopy.

**RESULTS**

Cell isolation of the left-lateral liver segments yielded 6.5 to 7.7x10\textsuperscript{9} hepatocytes. Viability determination was based on the trypan blue exclusion test and ranged from 75 % to 97 %. During total body scanning (TBSc), radioactivity was only detected in the region of the remnant liver (n=5). Organ scanning (Osc) showed radioactivity at the implantation sites in the mesentery and proximal jejunum, in two transplanted animals after 1 and 3 months, respectively (n=9). Radioactivity at these sites (direct tissue counting, DTC) was respectively 10.4% and 42.0% of radioactivity of the respective samples of the remnant liver. Radioactivity at the remaining injection sites in these animals, as well as in the other animals, showed only minimal or not elevated radioactivity when compared to control (sites injected with saline).

Histological examination showed clusters of morphologically normal hepatocytes at the implantation sites in the mesentery, bowel wall and stomach in all transplanted animals. Within these clusters of hepatocytes, sinusoid-like structures and ductular differentiation were observed. The hepatocytes injected into the subserosa of the bowel wall were identified deep in the submucosal layer in all transplanted animals (figures 1+2). On microscopical examination, hepatocytes could be detected in the pancreas only occasionally, in two transplanted animals. In the spleen, no hepatocytes could be detected in any of the animals.
Figure 1.
Histological appearance of hepatocyte clusters (arrows) at implantation sites of hepatocytes in small bowel wall.

Figure 2.
Histological appearance of hepatocyte clusters (arrows) at implantation sites of hepatocytes in mesentery.
DISCUSSION

In the present study the feasibility of hepatocyte transplantation in stomach and bowel walls was assessed in pigs. Liver cell transplantation would offer considerable advantages to whole (or auxiliary) liver transplantation, especially when it comes to substitution of one enzyme function such as in several, inherited metabolic liver diseases. One of the important issues in this field is finding the optimal route for transplantation and exploring the limits to the number of hepatocytes that can be transplanted. Clearly, only the possibility of transplanting large quantities of liver cells with immediate function would enable the use of hepatocyte transplantation in acute liver failure.

The majority of studies on hepatocyte transplantation and its morphologic, cytokinetic and metabolic aspects have been performed in rats and mice. Most research groups have chosen the spleen as the preferred acceptor site in rats or the recipient liver after intraportal injection of the hepatocytes. Clinical use of techniques of hepatocyte transplantation however would require demonstration of function in a large animal prior to application in humans. Only a few studies have been performed on hepatocyte transplantation in large animals, such as in pigs, and these studies mainly focus on intraportal and intrasplenic transplantation(1,3,5).

A consideration in choosing the small bowel wall and stomach as implantation sites is the embryonic origin of the liver in the foregut and the hepatotrophic stimulus implanted hepatocytes may receive when implanted in the area of portal venous drainage. An additional therapeutic option in case of successful hepatocyte transplantation in the stomach wall would be the possibility of endoscopic access to deliver therapeutic hepatocyte injections.

In the present report, autologous hepatocytes were transplanted in pigs, using the spleen, the pancreas, mesentery, stomach and small bowel wall as implantation sites. Although large numbers of hepatocytes were injected into the parenchyma of the spleen, while the afferent and efferent vessels were clamped, no hepatocytes were found after 1-3 months implantation. Presumably, most hepatocytes were lost due to embolization of the cells through the efferent vessels of the spleen. In contrast, the mesentery, stomach and small bowel wall showed evident take of hepatocytes, as detected by scintigraphy and microscopical examination. The normal morphology and the fact that in some animals these hepatocytes show uptake of HIDA-Tc$^{99}$ suggest that these hepatocytes are capable of function. A remarkable finding was the migration of hepatocytes into the submucosa directly adjacent to the mucosal layer of the bowel wall and around nerves.

In conclusion, these data demonstrate that in pigs, the mesentery and small bowel wall provide more favorable sites for transplantation of hepatocytes, compared to the pancreas or the spleen.
References


