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Autogenous hepatic tissue transplantation into the omentum in a novel ectopic liver regeneration murine model

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ABSTRACT

Background: Liver regeneration involves hyperplasia and hypertrophy of hepatic cells. The capacity of macroscopic liver tissue to regenerate in ectopic sites is unknown. We aim to develop a novel *in vivo* model of ectopic liver survivability and regeneration and assess its functionality.

Methods: Adult male Sprague–Dawley rats ($n = 23$) were divided into four groups: (1) single-stage (SS) group, wedge liver resection was performed, and the parenchyma was directly implanted into the omentum; (2) double-stage (DS) group, omentum pedicle was transposed over the left hepatic lobe followed by wedge liver resection along with omental flap; (3) Biogel + DS group, rats received intraperitoneal injection of inert polymer particles prior to DS; (4) Biogel + DS + portal vein ligation (PVL) group, Biogel + DS rats underwent subsequent PVL. Hepatobiliary iminodiacetic acid scintigraphy assessed bile excretion from ectopic hepatic implants.

Results: Histologically, the scores of necrosis ($P < 0.001$) and fibrosis ($P = 0.004$) were significantly improved in rats undergoing DS procedure (groups 2, 3, and 4) compared with the SS group. Biogel rats (Biogel + DS and Biogel + DS + PVL) demonstrated statistically increased scores of bile duct neoformation ($P = 0.002$) compared to those without the particles (SS and DS). Scintigraphy demonstrated similar uptake of radiotracer by ectopic hepatic implants in groups 2, 3, and 4.

Conclusions: Omental transposition provided adequate microcirculation for proliferation of ectopic hepatic cells after liver resection. Inert polymers enhanced the regeneration by promoting differentiation of new bile ducts. The ectopic hepatic implants showed preserved function on scintigraphy. This model provides insights into the capacity of liver parenchyma to regenerate in ectopic sites and the potential as therapeutic target for cell therapy in end-stage liver disease.

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Introduction

Liver failure is associated with high morbidity and mortality due to limited therapeutic approaches. Although liver transplantation is an effective therapy, its widespread use is restricted by donor organ shortage, immunosuppression side effects, technical complications, and socioeconomic constraints. Regenerative medicine is rapidly emerging with innovative techniques of cell therapy and organ engineering to overcome some of these limitations.

The liver has a remarkable regenerative ability involving mainly parenchymal cells and hepatocytes. The latter may be considered bipotent progenitor cells capable of dedifferentiation, rapid proliferation, and redifferentiation into new hepatocytes and cholangiocytes leading to hyperplasia and hypertrophy.[1] Nonparenchymal cells such as stellate cells, Kupffer cells, lymphocytes, and endothelial cells regulate this complex process by producing various growth factors including transforming growth factor-β, fibroblast growth factor, hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF).[2,3]

Although mechanisms involving liver regeneration have been extensively investigated, the capacity of macroscopic liver tissue to regenerate in ectopic sites is unknown. The omentum has been previously studied as a reservoir for proliferating splenic, renal, and pancreatic tissues.[4-6] Also, it has been evaluated as a site for engraftment of hepatocytes that could be potentially utilized in tissue engineering.[7] Clinically, the omentum is largely used to promote wound healing and revascularization of ischemic tissues such as omental patching in perforated peptic ulcer disease.[8,9] Herein, we aim to develop a novel *in vivo* model of ectopic liver regeneration by implanting autogenous macroscopic hepatic tissue into the omentum and assess its functionality.

Methods

Animals

Adult male Sprague–Dawley rats aged 8-12 wk weighing 539.5 ± 116.5 g (range, 328-815 g) received humane care in compliance with the ethics guidelines of Providence Hospital and Medical Centers, Michigan State University College of Human Medicine. The study was approved by our Institutional Animal Care and Use Committee. These animals were maintained in a standard animal laboratory with free activity and unlimited access to water and rodent chow. They were maintained in a temperature-controlled environment at 22°C-24°C with a 12-h light–dark cycle.

On day 0, the rats were randomly divided in four groups (Fig. 1):

Group 1-Single-stage (SS) ($n = 5$): wedge liver resection (WLR) of left hepatic lobe was performed. The parenchyma was divided into three small pieces and directly implanted into the omentum (Fig. 2);
Group 2-Double-stage (DS) ($n = 10$): an omentum pedicle was mobilized and transposed onto the liver (Fig. 2) followed by WLR along with the omental flap;
Group 3-Biogel + DS ($n = 4$): DS rats were pretreated with intraperitoneal injection of inert polymer (Biogel P-60, 120 μmol/L; Biorad Lab, Richmond, CA) 10 d prior omental transposition;
Group 4-Biogel + DS + portal vein ligation (PVL) ($n = 4$): Biogel + DS rats underwent PVL 15 d prior to harvest in an attempt to augment ectopic hepatic tissue implantation.

Surgical procedures

All procedures were performed under standard sterile conditions. After appropriate anesthesia was induced, the abdomen was shaved, prepped, and draped in the usual sterile fashion using alcohol-based solution.

Group 1
A 5-cm midline incision was performed and dissected to the fascia. The abdomen was entered, and the left hepatic lobe exposed (Fig. 2A). The lobe was partially transected with electrocautery, and hemostasis was ensured (Fig. 2B). The hepatic tissue was then sutured into the omentum (Fig. 2C) using 4-0 nonabsorbable polypropylene sutures (Prolene; Ethicon Inc, Somerville, NJ) to facilitate identification during harvest of specimens. The fascia was closed with interrupted 3-0 absorbable braided sutures (Vicryl; Ethicon Inc), and the skin was closed with 4-0 absorbable braided sutures (Vicryl).

Group 2
In this group, rats underwent a two-stage approach. The first procedure consisted of mobilization of the omentum and transposition onto the left hepatic lobe (Fig. 2) in an attempt to allow the omentum to incorporate onto the left lobe. On postoperative day 15, the rats underwent WLR in a similar fashion as described for group 1.

Group 3
In this group, the rodents were injected with 5 cc of polydextran particle slurry (Biogel P-60, 120 μmol/L). The slurry was diluted 1:1 with normal saline, 24 h before intraperitoneal injection (Fig. 3). After 10 d, the animals underwent the same protocol as the DS group.

Group 4
In this group, the animals underwent similar protocol as group 3 followed by PVL. On postoperative day 15, the rats were euthanized, and specimens were collected. This timeframe was allotted to enhance possible hypertrophy of the hepatic implant after PVL. Under general anesthesia, a subcostal incision was made to avoid midline adhesions. The small bowel and proximal transverse colon were mobilized and retracted medially to expose the portal triad. Circumferential dissection of the portal vein was performed to obtain proximal and distal vascular control. The vein was ligated with 2-0 silk (Silk, Ethicon Inc) sutures and transected. The abdomen was closed as previously described.

Anesthesia

Anesthesia was induced with Ketalar (90 mg/kg; Pfizer Inc, New York, NY) and Rompun (5 mg/kg; Bayer Inc, Shawnee