Whole Fetal Liver Transplantation—A New Approach to Cell Therapy

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We recently developed a novel rat model for liver repopulation, heterografting of microliver slices, aimed at overcoming the limitations inherent in both whole liver and hepatocyte transplantations. The aim of the present study was to evaluate the potential of whole fetal liver transplantations to survive and differentiate within the adult liver, using the adult liver slice transplantation model. Embryonic day 14 whole fetal livers from dipeptidyl peptidase IV/+/+ wild-type Fischer 344 rats were transplanted into the livers of dipeptidyl peptidase IV−/− mutant rats. Adult hepatic markers, dipeptidyl peptidase IV, albumin, glycogen, and proliferation cell nuclear antigen—proliferation cell nuclear antigen (PCNA) were assessed in the transplanted liver tissue by immunohistochemistry. Two groups of 9 rats each were transplanted with 3 fetal livers per recipient. Two months later the rats were sacrificed and the markers were detected in the transplanted tissues. In conclusion, the results of this study raise the possibility that fetal liver transplantation could serve as a model for genetic metabolic liver diseases. (Liver Transpl 2005;11: 929-933.)

Liver transplantation provides a definitive cure for various liver diseases by replacing all of the liver cells.1 Although there are ongoing technical improvements to liver transplantation techniques (liver splitting, reduced size grafts, living donor liver transplantation), the procedure still carries considerable risks and limitations.2, 3 Moreover, the worldwide availability of organs for transplantation is very limited.4, 5 Hepatocyte transplantation is a potentially promising alternative to whole organ transplantation.6 However, cell therapy in humans is still not used widely due to the poor viability of cryopreserved cells, weak initial cell engraftment, and the lack of clinically safe procedures that can ensure a growth advantage for the transplanted cells.

We recently proposed an alternative strategy for hepatocyte transplantation and reported successful liver repopulation following transplantation of adult liver slices.7 This method, heterografting of liver slices, serves as a vehicle for the introduction of cells into the liver, obviates the need to isolate cells, and provides them with artificial conditions for growth and development.7 To improve this strategy, we designed a study to evaluate the potential role of the transplantation of rat embryonal day 14 (E14) whole fetal livers.

Fetal hepatocytes have several advantages over adult liver cells. They offer a potentially unlimited source of cells for hepatocyte replacement,8 and they also have the capacity to differentiate into hepatocytes and bile duct epithelium.8, 9 When the repopulation capacity of E14 fetal liver epithelial progenitor cells transplanted into normal liver was compared to adult hepatocytes, 3 important differences were noted: 1) fetal liver epithelial progenitor cells continued to proliferate for up to 6 months following transplantation, whereas adult hepatocytes ceased proliferation within the first month; 2) the number and size of clusters derived from fetal liver epithelial progenitor cells gradually increased over time, compared to transplanted mature hepatocytes, which decreased over time; 3) fetal liver epithelial progenitor cells differentiated into hepatocytes when engrafted into the liver parenchyma and into bile duct epithelial cells when engrafted into the vicinity of the host bile ducts, compared to adult hepatocytes, which did not form bile duct structures.9

Thus, we conducted a study to investigate the outcome of the transplantation of fetal livers using a heterografting technique originally developed for adult liver slices.7

Abbreviations: DPP-IV, dipeptidyl peptidase IV; PCNA, proliferation cell nuclear antigen.

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Materials and Methods

Animals

Donor Rats
Female wild type Fischer 344 rats served as donor rats for these studies (9). DPP-IV+/+ female rats with known durations of pregnancy (E14) were obtained from the Tel Aviv Sourasky Medical Center Animal Facility. By convention, the first day of gestation was defined as day 0.

Recipients
Male Fischer 344 lacking the canalicular enzyme dipeptidyl peptidase IV (DPP-IV-/-) served as recipient rats. The Animal Use and Care Committee of the Tel Aviv Sourasky Medical Center approved the study. All study animals received humane care according to the criteria detailed in the “Guide for the Care and Use of Laboratory Animals” (NIH publication 86-23 revised 1985).

Isolation of E14 Fetal Rat Livers
Livers were removed from E14 embryos and placed in ice cold, Ca²⁺/Mg²⁺-free Hank’s Balanced Salt Solution containing 0.8 mmol/L MgCl₂ and 20 mmol/L HEPES (Sigma Chemical Company, Israel), pH 7.3 (mod Hank’s Balanced Salt Solution).

Surgical Procedure for Transplantation of Fetal Livers
The rats were operated on under general anesthesia (ketamine 90 mg/kg and xylazine 10 mg/kg). The fetal livers (3 livers per recipient) were grafted to the liver tissue of the recipients. The donor fetal livers were kept in Hank’s Balanced Salt Solution until used. At this stage, the recipient rat liver was stabbed using a No. 20 scalpel on the undersurface of its left lobe. The stab wound was administered on a horizontal plane in the liver tissue, to a depth that was sufficient to encompass the donor fetal livers. The fetal livers, situated on the blade, were embedded in the slit in the recipient liver until they could no longer be seen. The wound site was then sutured with reabsorbing sutures.

DPP-IV Histochemical Staining
Serial cryosections of the liver region containing the grafted slices were prepared (5 μm thick) and fixed in 95% ethanol: acetic acid (99:1) for 5 minutes at −200°C, then fixed for 5 minutes at 40°C in 95% ethanol. The sections were air dried and incubated with the substrate solution (Gly-Pro 4-methoxy-β-naphthylamide (Sigma, St. Louis, MO) in dimethyl formamide and fast blue BB salt) for 20 minutes at 37°C. They were then washed and incubated with 0.1 mol CuSO₄ for 2 minutes. DPP-IV activity was manifested as orange-red canalicular staining on hepatocytes.

Immunohistochemistry
Paraffin-embedded liver sections were stained with antibodies to proliferating nuclear antigen (PCNA) (Dako, Glostrup, Denmark) and to rat albumin. Paraffin sections of 4 μm were mounted on poly-L-lysine glass slides and deparaffinated with xylene and graduated ethyl alcohol, then rehydrated with Tris-buffered saline, pH 7.4. Before incubation with the primary antibody, sections were incubated twice for 5 minutes in a microwave oven in 10 mmol/L citrate buffer. The sections were incubated for 60 minutes with the primary antibodies. The slides were washed 3 times with Tris-buffered saline, incubated with a biotinylated mouse secondary antibody (Zymed Laboratories, San Francisco, CA), and washed and labeled with peroxidase-conjugated streptavidin (Zymed Laboratories). Staining was visualized using 3,3’-diaminobenzidine (DAB) substrate.

Glycogen Staining
Glycogen granules were detected with a Periodic Acid Schiff (PAS) kit (Sigma, St. Louis, MO), in accordance with the manufacturer’s instructions.

Results
Nine mutant DPP-IV−/− Fischer 344 rats served as recipients. Fetal livers from E14 wild-type DPP-IV+/+ were used for transplantation. Three whole E14 fetal livers were transplanted into the liver of each adult recipient as previously described for transplantation of adult liver slices. The experiments were...
Figure 2. Detection of proliferation and maturation in the transplanted liver tissue. (A, B) Albumin, (C, D) PCNA, and (E, F) glycogen expression in (A, C, E) rat E14 fetal livers transplanted into (B, D, F) normal adult livers. Livers from E14 Fisher rats DPP-IV<sup>+/+</sup> were transplanted into the liver of mutant DPP-IV<sup>−/−</sup> male rats. Two months later, the livers were removed, and paraffin sections were stained with antibodies to albumin and PCNA (Dako) (positive staining is brown in A, B, C, D). Glycogen expression was detected by histochemical staining using a PAS staining kit (violet, E, F).
repeated twice. The rats were sacrificed 2 months after transplantation. DPP-IV enzymatic activity was assayed to detect transplanted liver tissue, as well as to determine the type and degree of differentiation of the transplanted tissue, since at E14 DPP-IV is not yet expressed (Fig. 1). The transplanted fetal tissue expressed DPP-IV, while the surrounding adult hepatocytes did not (Fig. 1).

For further assessment of the maturation and survival of the transplanted livers, albumin and glycogen expression were determined (Fig. 2). Albumin was expressed in most of the transplanted cells, while glycogen granules, which appear in rats after birth, were present in scattered groups of cells (Fig. 2). PCNA, a proliferation marker, was detected in most of the transplanted fetal tissue, but not in the surrounding adult hepatocytes (Fig. 2). The recipient rats were alive and doing well throughout the study period, reflecting the safety of the procedure.

In the past, fragments of liver have been transplanted orthotopically in various extrahepatic sites with limited success.10-12

Discussion
The results of this study demonstrate, for the first time, that fetal liver cells need not be isolated or preconditioned before transplantation, but they can be transplanted in the form of a whole fetal liver. Moreover, this is the first report that adult livers can provide a suitable environment for the development of fetal livers that were transplanted with intact capsules.

Whole liver transplantation has become standard treatment for end-stage liver disease over the past decade. However, broader application of this procedure is limited by the small number of available organ donors.1 Thus, the development of effective methods for hepatocyte transplantation is a major goal of current research, because of the potential to treat viral-induced end-stage liver failure, as well as genetic liver diseases, such as hereditary hemochromatosis, Wilson’s disease, and alpha-1-antitrypsin deficiency.

The results of numerous laboratory studies have shown that hepatocyte transplantation can serve as an alternative to organ transplantation in patients with life-threatening liver disease. However, there is a major obstacle to the transplantation of adult hepatocytes to the liver or spleen. To achieve proliferation of the transplanted hepatocytes, 2 conditions must be met: (1) proliferation of resident hepatocytes has to be arrested with toxic, carcinogenic substances (retorsine), and (2) transplanted hepatocytes require a strong mitogenic stimulus, usually partial hepatectomy, to proliferate.13 Obviously, this approach is unsuitable for human cell therapy with hepatocytes. Safer, practical methods have to be developed.

We recently proposed an alternative to hepatocyte transplantation and reported significant success in the transplantation of adult liver slices.7 This method, heterografting of liver slices or topo-transplantation, is a vehicle for the introduction of cells into the liver that is devoid of the disadvantages of isolated cells, which require artificial conditions for their growth and development.7

In the present study, we investigated further the potential of tissue heterografting by using early rat fetal livers rather than adult liver slices. We hypothesized that fetal hepatocytes may have several advantages over adult liver cells in that they could differentiate into hepatocytes and bile duct epithelium8 and their repopulation capacity might be better than that of adult hepatocytes.1,6,8 The results of the study show that E14 transplanted fetal livers have a high degree of proliferative activity, as demonstrated by PCNA staining. Moreover, we have also shown that the transplanted tissue differentiates as demonstrated by DPP-IV and glycogen. From the safety perspective, it is noteworthy that all study rats survived without any major complications.

In conclusion, we believe that transplantation of fetal livers could provide a useful alternative to current methods that entail the transplantation of isolated cells.

References


