
A Novel Method for Liver Repopulation: Heterografting of Micro-Liver Slices in a Rat Model

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The field of hepatocyte transplantation is developing, with encouraging results. However, current approaches are still unsuitable for human cell therapy, and safer and more applicable methods need to be developed. We recently successfully transplanted pieces of liver tissue (slices), cut from a wild-type Fischer 344 dipeptidyl peptidase IV (DPP IV)–positive rat and introduced into the liver of a DPP IV–deficient Fischer 344 rat. One month after the procedure, positive DPP IV enzymatic activity was detected in transplanted liver slices. These results suggest that transplantation of tissue slices is feasible and safe and could serve as a promising alternative to hepatocyte transplantation. (Liver Transpl 2003;9:421-424.)

In the past decade, whole-liver transplantation has become standard treatment for end-stage liver disease and genetically metabolic liver diseases. Nevertheless, this approach also presents disadvantages for routine use in humans because of technical problems in the isolation and preservation of transplanted hepatocytes and the limited growth of transplanted hepatocytes in the recipient liver. Methods for hepatocyte isolation use calcium chelators to block cadherin-mediated cell-cell adhesion in conjunction with such enzymes as collagenase that cause detachment of cells from their underlying basement membrane. Therefore, these isolation procedures induce a high rate of detachment-induced apoptosis, termed anoikis. An elevated rate of apoptosis also is observed after thawing of cryopreserved hepatocytes.

In animal models, repopulation of the liver by transplanted hepatocytes was obtained in models of continuous destruction of resident hepatocytes (urokinase type plasminogen-activator transgene expression in the liver), in mice with tyrosinemia, or by blocking resident hepatocytes with retorsine and conferring a growth advantage to transplanted cells by partial hepatectomy or triiodothyronine injection. However, cell therapy in humans as routine treatment for liver disease still has a long way to go because of poor initial cell engraftment and difficulty establishing safe clinically applicable procedures that will cause a survival advantage for transplanted hepatocytes. Thus, safer and more applicable methods must be developed, and the aim of the present study is to determine the possibility of heterografting microthin liver slices in a 344 Fischer rat model.

Materials and Methods

Animals

Wild-type Fischer 344 male rats and male mutant Fischer 344 rats that lack the canalicular enzyme dipeptidyl peptidase IV (DPP IV−) were used for these studies. Animals were maintained in the animal facility of the Tel Aviv Sourasky Medical Center (Tel Aviv, Israel) on a standard rat chow diet with 12-hour light cycles. Use of animals was in accordance with The National Institutes of Health (NIH) Policy on the care and use of laboratory animals and approved by the Animal Use and Care Committee of the Tel Aviv Sourasky Medical Center. All animals received humane care according to criteria in Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1985).

Surgical Procedure

We used 18 mutant DPP IV− Fischer 344 rats as recipients and 6 wild-type Fischer 344 rats (300 to 350 g) as donors. Rats were operated on under general anesthesia (ketamine, 90 mg/kg; xylazine, 10 mg/kg). Very thin liver slices (86 ± 12 by 296 ± 40 by 12 ± 5 cells) were harvested to serve as a donor liver, then grafted into the liver tissue of recipients (Fig. 1). The donor rat liver capsule was peeled off first to expose the liver parenchyma (Fig. 1A). Very fine liver tissue was tangentially harvested using a sharp no. 20 scalpel blade (Fig. 1B). The donor tissue liver slide was kept wet on a gauze wet with 12-hour light cycles. Use of animals was in accordance with The National Institutes of Health (NIH) Policy on the care and use of laboratory animals and approved by the Animal Use and Care Committee of the Tel Aviv Sourasky Medical Center. All animals received humane care according to criteria in Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23, revised 1985).
saline until used (Fig. 1B). At this stage, the recipient rat liver, on the undersurface of its left lobe, was stabbed using the same no. 20 scalpel (Fig. 1C). The stab wound entered the liver tissue on a horizontal plane parallel to the longitudinal axis of the lobe (Fig. 1C) to a distance sufficient to host the donor rat liver slice. If bleeding occurred, the site was packed with Gelfoam and held under sufficient digital pressure until dried. The slice, lying on the blade, was embedded inside the recipient site until fully hidden (Fig. 1D). The opening of the stab site then was sutured (Fig. 1E).

The transplantation site was a constant one and marked with silk sutures. The specific liver segment was harvested including two layers of recipient tissue, with the transplanted slice sandwiched in between.

DPP IV Histochemical Staining
Serial cryosections of the liver region containing the grafted slice were prepared (5-μm thick) and fixed in 95% ethanol–acetic acid (99:1) for 5 minutes at −20°C, then fixed for 5 minutes at 4°C in 95% ethanol. 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. Sections then were washed and incubated with 0.1 mol/L of CuSO₄ for 2 minutes. DPP IV activity was viewed as orange-red canalicular staining on hepatocytes.

Results and Discussion
Before transplantation of slices to a large group of animals, we conducted preliminary studies to determine the optimal thickness of the transplanted slice. Thickness was determined by measuring with a caliper, and we observed that the slice “take” depended almost entirely on the thickness of the slice.

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<th>No. of Transplant Recipients</th>
<th>Date Killed After Transplantation</th>
<th>No. of Rats Positive for Transplanted Slice</th>
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A group of 18 rats underwent transplantation with slices from wild-type Fischer rats, and 6 rats were killed at 2 days, 2 weeks, and 1 month after transplantation. The liver region that contained the grafted slice was frozen. All 18 rats were well and alive throughout the study period, thus representing the safety of this protocol. Results of transplantation experiments are listed in Table 1. At each time, DPP IV enzymatic activity was detected in 2 of 6 transplant recipients (Table 1; Fig. 2). The interface between the transplanted donor liver tissue was well observed (Fig. 2). In the 12 rats negative for DPP IV activity, limited tissue necrosis was noted in 7 animals (Table 1). However, it did not affect the general condition of the rat. We were unable to identify the transplanted tissue in 5 other rats because of severe adhesions and technical problems (Table 1).

We believe the process is similar to skin grafting as a plastic surgery technique for closure of full-thickness skin defects. To prevent graft necrosis, it was shown that by reducing graft thickness, “take” will be enhanced. Neovascularization was needed to emerge from the host bed and penetrate the graft for the graft to survive. Penetration was made easier when graft thickness was reduced, not letting the most remote graft cells become ischemic. Later, the same principle of complex tissue grafting was applied to other tissue, such as fat, fascia, cartilage, and bone.

The pathophysiological process of skin graft “take” is characterized by a three-phase process that occurs almost simultaneously. In the first phase, plasmatic inhibition, serum ingredients diffusely nourish the cells. In the second inoculation phase, existing blood vessels from the recipient bed transfer blood cells to the amputated blood vessel openings in donor tissue in a noncirculatory fashion. In the third neovascularization phase, new blood vessels from the recipient bed penetrate the donor tissue, creating arterial and venous anastomoses to establish blood circulation through the grafted tissue.12 In our opinion, the same principle of three pathophysiological phases, characterizing skin graft “take,” can be applied to parenchymal tissue grafting, suggesting that donor liver slices can be successfully grafted in a well-vascularized recipient liver bed.

Liver slices also have been used by other groups, mainly for ex vivo basic science research.13 However, to the best of our knowledge, this study is the first to propose transplanting the slices for repopulation of the liver.

This study suggests that the slice approach is novel, safe, and simple. We believe it could be widely applicable for transplantation of cells, ex vivo gene transfer, and basic science research.

References