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Liver Cell Transplantation For Primary Hyperoxaluria

b. SPECIFIC AIMS

Purpose of the following three specific aims is to optimization of the preparative regimen for massive repopulation of Agxt$^{-/-}$ recipient mice by wildtype LacZ-transgenic (ROSA)26 donor mouse hepatocytes: The optimum temporal relationship between hepatic irradiation, hepatocyte transplantation and mitotic stimulation needs to be established.

Specific aim b.1. **We will determine empirically the HIR dose** that leads to (i) maximum engraftment and (ii) replacement of host hepatocytes by the donor cells.

Specific aim b.2. We will delineate the optimum temporal relationship between HIR and hepatocyte transplantation, and mitotic stimulation of the engrafted hepatocytes (by administration of a recombinant adenoviral vector expressing human hepatocyte growth factor (HGF). Once this information is available, we will use infusion of recombinant HGF instead of gene transfer. Extent of liver repopulation will be quantified by histochemical staining of the liver, biochemical analysis, quantitative PCR, Western blot and AGT assay.

Specific aim b.3. **We will evaluate the metabolic consequences of hepatic repopulation** from two view points: (a) extent of amelioration of PH1 will be assessed by analysis of urinary glycolate and oxalate excretion and oxalate crystals in urine sediments. We will also determine whether hepatic repopulation can protect the mice against nephrocalcinosis and urinary bladder epithelial injury after increase of oxalate load by ethylene glycol administration. (b) General synthetic and excretory functions of the repopulating hepatocytes will be evaluated by measurement of serum albumin and bilirubin levels, respectively. Two series of experiments will be performed to evaluate what fraction of the mutant hepatocyte mass must be replaced by transplanted cells for therapeutic benefit:

Specific aim b.1. **We will determine empirically the HIR dose** that leads to (i) maximum engraftment and (ii) replacement of host hepatocytes by the donor cells.

Engraftment of donor hepatocytes is a critical step that determines the success of hepatocyte transplantation. Rapid and efficient integration of donor cells would enable prompt liver repopulation of these cells in response to selective proliferative stimuli offered by a preparative regimen. We demonstrated earlier that hepatic irradiation (HIR) in combination with a variety of hepatotrophic growth signals, such as partial hepatectomy and hepatocyte growth factor, can be used as a preparative regimen for liver repopulation of transplanted hepatocytes.

We hypothesized that a major barrier to initial engraftment of hepatocytes arriving into the liver through the portal circulation is the integrity of the hepatic sinusoidal endothelium. HIR may enhance engraftment by transiently disrupting the integrity of the sinusoidal endothelium. To determine the optimum temporal relationship between HIR and hepatocyte transplantation, we investigated the effects of HIR on the initial engraftment of transplanted dipeptidyl peptidase IV (DPPIV)–positive hepatocytes in congeneic DPPIV-deficient rats. Transmission electron microscopy revealed that HIR had induced apoptosis of hepatic sinusoidal endothelial cells (SEC) within 6 hours of HIR. In another 24 hr, the cells showed dehiscence. The dysfunction of the sinusoidal endothelium was also confirmed by reduced hyaluronic acid clearance. The number of viable transplanted hepatocytes integrated in the parenchyma following HIR at doses as

low as 10 Gy. Thus, HIR clearly enhanced initial engraftment of hepatocytes at even low doses. However, proliferation of the engrafted hepatocytes required higher dose of hepatic irradiation (20-50 Gy), suggesting that the long-term effect of HIR on hepatocytes required a higher radiation dose than did transient disruption of endothelial cells. Based on our findings, in subsequent studies in the Agxt-/- mice we transplanted the hepatocytes 24 hours after HIR.

Specific aim 2. As we mentioned in our preliminary studies that to circumvent the need for partial hepatectomy, we were exploring alternative methods for providing mitotic stimuli to the engrafted hepatocytes. Partial hepatectomy induces compensatory hyperplasia of the liver by releasing several cytokines, including epidermal growth factor (EGF) and hepatocyte growth factor (HGF).We hypothesized that a high level of HGF expression would serve as a substitute for partial hepatectomy. To test this, we generated a recombinant adenoviral vector expressing human HGF (Ad-HGF). Mice were injected with $1X10^{11}$ viral particles through the tail vein, 2 days after HIR to generate high hepatic and plasma HGF concentrations. ELISA and Western blot, respectively, showed plasma and hepatic HGF levels that were comparable with mouse HGF levels after partial hepatectomy. Hepatocytes ($1X10^{7}$) were transplanted 1 day after HIR as described above. A control group received (a) HIR plus an adenovector expressing the green fluorescent protein (Ad-GFP), (b) Ad-hHGF alone or (c) HIR alone before hepatocyte transplantation. Mice from each group were sacrificed at various time intervals after transplantation. At week 1, in all groups, hepatocytes were engrafted largely as single cells, primarily in the periportal region (Zone 1). After 20 weeks, livers of mice receiving HIR alone or Ad-HGF alone still contained the engrafted hepatocytes predominantly as single cells or clusters of 2-3 cells. In contrast, when both preparative HIR and Ad-HGF were used, the engrafted hepatocytes exhibited some proliferation in 4 weeks and formed large clusters in 8 weeks. By 20 weeks, 90-95% of the host hepatocytes were replaced by the β-galactosidase positive engrafted hepatocytes.

c.2.3. Effect of liver repopulation on urinary oxalate excretion in Agxt$^{-/-}$ mice: As we reported in our preliminary studies, in extensive repopulation of the liver, urinary oxalate excretion was reduced to near-normal levels. However, for potential clinical application, it is necessary to determine the level of hepatic repopulation that is needed for significant reduction (rather than complete normalization) of urinary oxalate excretion, so that after partial repopulation of the liver, the PH1 patients could be managed by medical procedures that are being developed for reducing hyperoxaluria.

To quantify the relationship between liver repopulation and decline of hyperoxaluria, we performed two series of experiments. In one series, Agxt$^{-/-}$ mice were transplanted with hepatocytes from wildtype LacZ transgenic mice (ROSA26). In a second series, hepatocytes from Agxt$^{-/-}$ mice were transplanted into the wildtype ROSA26 recipients. Animals in each group were sacrificed at various time points after transplantation. Urine was collected overnight for each mouse before sacrifice. The level of repopulation was determined by staining liver sections for β-galactosidas-positive activity. In the Agxt$^{-/-}$ recipients, clusters of β-galactosidas-positive hepatocytes increased in size in a time-related manner. On the other hand, in the ROSA26 recipients, clusters of β- galactosidas -negative cells grow in the recipient liver over time (Fig. 1a). The percentage of Agxt-/- hepatocytes remaining in the liver in each recipient was plotted against the urinary oxalate/creatinine ratio (Fig. 1b). Our results indicate that there is no sharp threshold for the level of repopulation that is required for reducing urinary oxalate excretion. Rather, there is a progressive decrease as the mutant hepatocytes are depleted with time.

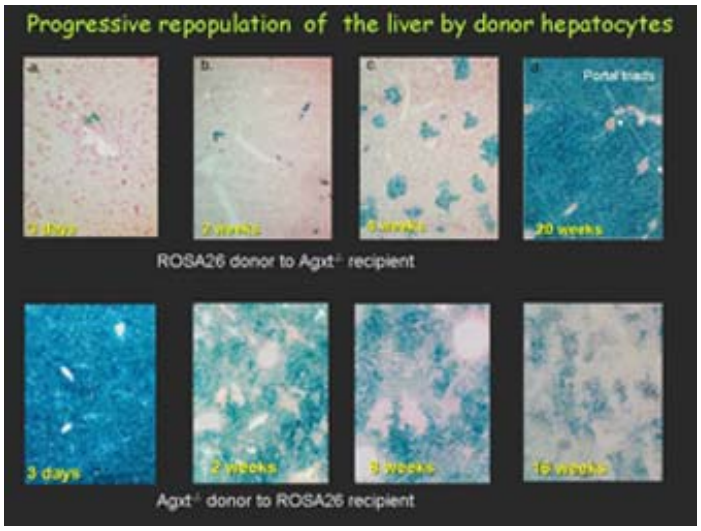

Figure 1a. Liver sections stained for β-galactosidase activity after hepatocyte transplantation. The top row shows livers of Agxt$^{-/-}$ mice transplanted with hepatocytes from wildtype LacZ transgenic mice (ROSA26), blue clusters indicate donor cells. The bottom row shows livers from ROSA26 recipients transplanted with hepatocytes from Agxt$^{-/-}$ mice, the white clusters indicate donor cells. Time intervals after transplantation are indicated.

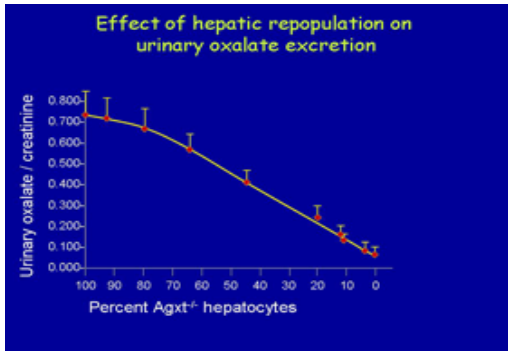

Figure 1b. Correlation of urinary oxalate to creatinine ratios and the percent of Agxt$^{-/-}$ hepatocytes remaining in the liver. Hepatocytes were transplanted into recipient mice undergoing preparative HIR and Ad-HGF administration as in Figure 1a. Before sacrifice, each recipient underwent urine collection for analysis of creatinine and oxalate. The proportion of Agxt$^{-/-}$ cells (β-galactosidase-negative) was determined by computerized image analysis. Means ± SD are shown. N=6.