Evaluation of transplanted hepatocytes using HIDA scintigraphy

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Background: Hepatocyte transplantation has generated interest because of potential clinical application in enzyme deficiency disorders and acute hepatic failure. Ex-vivo HIDA scintigraphy has been used to assess graft survival after hepatocyte transplantation. The present study evaluates in-vivo 99mTc-HIDA scintigraphy to assess graft function after hepatocyte transplantation. Methods: Rat hepatocytes were isolated by a modified collagenase digestion technique and injected intrasplenically into 6 syngeneic rats; 4 control rats received intrasplenic saline injections. In-vivo HIDA scintigraphy and histological evaluation were done 90 days after transplantation. Results: Five of the six rats in the study group showed prompt and progressive accumulation of HIDA in the spleen. Histological examination showed presence of hepatocytes in the splenic red pulp. None of the control group animals had splenic uptake of HIDA. Conclusion: HIDA scintigraphy may be a useful modality for assessment of graft function after intrasplenic hepatocyte transplantation. [Indian J Gastroenterol 2001;20:177-179]

Key words: Hepatocyte survival, liver scan

Isolated rat hepatocytes transplanted into syngeneic rat spleens have been shown to survive, proliferate, get organized in cords, rosettes and pseudocini, and continue their metabolic activity. Various studies have demonstrated the presence of glycogen, albumin, glucose 6-phosphatase and urea cycle enzymes in the transplanted hepatocytes. The functional viability of these transplanted hepatocytes has been assessed by measurement of albumin secretion, bilirubin glucuronidation, and activities of urea cycle and cytochrome P450 systems.

It is important to have a reliable and noninvasive technique to monitor graft survival after hepatocyte transplantation (HT). Cuervas-Mons et al and Woods et al used static 99mTc-HIDA scintigraphy to demonstrate viability of transplanted hepatocytes. The present study was conducted to evaluate whether in-vivo 99mTc-HIDA scintigraphy can be used to assess graft survival after HT.

Methods

The protocol for the study was approved by the Ethics Committee of our institution. Adult inbred Fisher rats, bred in our laboratory, were used as hepatocyte donors and recipients. The animals were housed in controlled temperature rooms and received standard rat chow and water ad libitum. A single intraperitoneal injection of a broad-spectrum cephalosporin was given preoperatively.

Hepatocyte isolation and transplantation

Hepatocytes were isolated by a two-step collagenase digestion method described previously. The portal vein of an anesthetized donor rat was cannulated and the liver was perfused with a calcium-free perfusion buffer containing ethylene diamine tetraacetate (EDTA) at a flow rate of 30 mL per minute. This was followed by perfusion with a collagenase buffer (type I collagenase 0.5 mg/mL in calcium-free Hank’s solution) at an infusion rate of 30 mL/min for 10 minutes. The soft and friable liver was excised and gently minced in 3 mL of fetal calf serum. The initial cell suspension obtained was filtered through a 100 µm nylon sieve, washed twice in minimum essential medium (MEM) and centrifuged at 500 rpm for 3 minutes. The cell pellet was resuspended in MEM at 4°C (16 x 10⁶ cells/mL) and stored in an ice bath. The trypan blue exclusion test was performed to assess cell viability.

Within 2 hours of hepatocyte isolation, a midline celiotomy was performed under ketamine anesthesia in 12 syngeneic rats and 0.5 mL of hepatocyte suspension was injected directly into the splenic parenchyma. Hemostasis was achieved by tamponade of the puncture site with a cotton tip. The peritoneal cavity was washed with normal saline and the abdomen closed in a single layer using 4-0 catgut. Four control animals underwent laparotomy and injection of 0.5 mL normal saline into the splenic parenchyma. There were 6 late postoperative deaths (>4 weeks following laparotomy) in the transplanted group. These animals were excluded from the study.

99mTc-HIDA scintigraphy

Six surviving rats with HT and the four control rats underwent in-vivo HIDA scan 90 days after the procedure. Freshly prepared 0.1 mCi of 99mTc-HIDA was injected as a bolus in the inferior vena cava of anesthetized rats after midline laparotomy. Planar dynamic images were acquired using a gamma camera with a low-energy
all-purpose (LEAP) pinhole collimator and a zoom factor of x2 for 180 seconds. The maximum in-vivo hepatic uptake of 99mTc-HIDA in rats is between 1 min and 3 min after intravenous administration. In each rat, scintigraphic images of anatomical distribution of HIDA uptake were displayed on a monitor in time frames of 6 seconds. The spleen was subsequently excised and placed under the camera for 60 seconds to record the uptake. This was done to rule out any confusion of splenic uptake with the liver uptake on in-vitro scanning.

Histological assessment

The rats were sacrificed by exsanguination under anesthesia after the HIDA scintigraphy. The excised spleens of all animals were fixed in 10% buffered formaldehyde and embedded in paraffin. 6-μ thick sections were stained with hematoxylin and eosin and by the periodic acid-Schiff technique (PAS) after diastase digestion.

Results

The trypan blue exclusion test showed 80%-85% viable cell yield using the isolation procedure described.

In the control group (n=4), immediately following intravenous administration of 99mTc-HIDA, high activity was noted over the heart, lungs and aorta, which rapidly decreased to background levels by 30 seconds. Hepatic uptake appeared at 1 minute and progressively increased over the next 2 minutes. There was no activity in the spleen in situ or after removal. Spleens of the control group showed normal splenic architecture and no PAS-positive granules on histological examination.

In 5 of the 6 HT rats, there was diffuse uptake of HIDA by the spleen at 1 minute, which increased progressively till 3 minutes post-infusion (Fig). The uptake in the spleen was simultaneous with the hepatic uptake. The spleen also showed activity after excision.

In one of the HT rats, no splenic uptake of HIDA was noted. Microscopic examination showed single or small groups of hepatocytes with microvesicular fatty changes. The cells were located in the splenic pulp. No nodule formation or sinusoidal architecture was discernible. PAS-positive granules suggesting presence of glycogen were noted in the transplanted hepatocytes.

Discussion

The present study demonstrates the feasibility of using in-vivo HIDA scintigraphy to assess graft viability after hepatocyte transplantation in the spleen. Uptake of HIDA by transplanted hepatocytes was demonstrated in 5 of 6 rats, 90 days following HT. The histological finding of hepatocytes in the splenic red pulp corresponded to the uniform splenic uptake seen on HIDA scintigraphy. None of the control group animals showed splenic uptake of HIDA.

HT has been applied in experimental studies to ameliorate enzyme deficiency states and to treat fulminant hepatic failure. Histological evaluation has been the gold standard for assessing graft survival. Woods et al. first demonstrated the use of HIDA scintigraphy to assess graft function after HT. Shortly following the administration of HIDA, the rats were sacrificed and the radioactivity in the excised spleen was compared to that in a blood sample. Radioactivity accumulation in the spleen of autografted rats was significantly greater than that in the control animals. Cuervas-Mons et al. also showed that ex-vivo HIDA scintigraphy can assess graft viability after intrasplenic HT. Vroomen et al. were the first to use in-vivo dynamic 99mTc-HIDA scintigraphy as a method for assessment of graft function after HT. They demonstrated a correlation between increased splenic uptake of HIDA and histological survival of intrasplenic donor hepatocytes. In our study, the survival and functional viability of transplanted hepatocytes was demonstrated by in-vivo HIDA scintigraphy. More importantly, the time-activity curves over the liver and spleen of transplanted rats were similar to each other and no other focus of HIDA accumulation was seen. The HIDA scintigraphy picture of uptake by the entire spleen corresponded well with the histological finding of diffuse presence of hepatocytes in the splenic red pulp.

Unlike histological and perfusion studies, HIDA scintigraphy is noninvasive. It is also safe, easy to perform and reliable for assessing graft survival after HT. Since the study can be repeated, HIDA scintigraphy may be an ideal modality to detect cell proliferation or assess rejection after allogenic transplantation.

References

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