Hepatic Irradiation Augments Engraftment of Donor Cells Following Hepatocyte Transplantation

Kosho Yamanouchi,1 Hongchao Zhou,1 Namita Roy-Chowdhury,2,3 Frank Macaluso,4 Liping Liu,5 Toshiyuki Yamamoto,5 Govardhana Rao Yannam,3 Charles Enke,6 Timothy D. Solberg,6 Anthony B. Adelson,7 Jeffrey L. Platt,8 Ira J. Fox,5 Jayanta Roy-Chowdhury,2,3 and Chandan Guha1,3*

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 have earlier demonstrated 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. In this study, we investigated the effects of HIR on engraftment of transplanted dipeptidyl peptidase IV (DPPIV)–positive hepatocytes in congenic DPPIV-deficient rats. HIR-induced apoptosis of hepatic sinusoidal endothelial cells (SEC) within 6 hours of HIR resulted in dehiscence of the SEC lining in 24 hours. Although there was no change of the number of Kupffer cells after HIR, colloidal carbon clearance decreased 24 hours post HIR, indicating a suppression of phagocytic function. DPPIV+ donor cells were transplanted 24 hours after HIR (0-50 Gy). There was an HIR dose-dependent increase in the donor hepatocyte mass engrafted in the liver parenchyma. The number of viable transplanted hepatocytes present in hepatic sinusoids or integrated in the parenchyma was greater in the HIR-treated group at 3 and 7 days after transplantation compared with the sham controls. Finally, we validated these rodent studies in cynomolgus monkeys, demonstrating that a single 10-Gy dose of HIR was sufficient to enhance engraftment of donor porcine hepatocytes. These data indicate that transient disruption of the SEC barrier and inhibition of the phagocytic function of Kupffer cells by HIR enhances hepatocyte engraftment and the integrated donor cell mass. Thus, preparative HIR could be potentially useful to augment hepatocyte transplantation. (HEPATOLOGY 2009;49:258-267.)

Abbreviations: ALT, alanine aminotransferase; ATPase, adenosine triphosphatase; DPPIV, dipeptidyl peptidase IV; ELISA, enzyme-linked immunosorbent assay; HA, hyaluronic acid; HIR, hepatic irradiation; HPF, high-power field; HT, hepatocyte transplantation; PVE, portal vein embolization; SEC, sinusoidal endothelial cells; TEM, transmission electron microscopy; TUNEL, terminal deoxynucleotidyl transferase-mediated nick-end labeling.

From the Departments of 1Radiation Oncology and 2Medicine, the 3Marion Bessin Liver Research Center, the 4Department of Anatomy and Cell Biology, Albert Einstein College of Medicine, Bronx, New York; and the Departments of 5Surgery, 6Radiation Oncology, and 7Radiology, University of Nebraska Medical Center, Omaha, NE; and the 8Departments of Surgery, Immunology, and Pediatrics, Mayo Clinic, Rochester, MN.

Received February 20, 2008; accepted August 4, 2008.

Supported by National Institutes of Health grants R01 DK64670 and R21/R33 CA121051 (to C.G.); R01 DK 039137 (to N.R.C.); R01 AI49472 (to I.J.F.); R01 DK48794 (to I.J.F.); and R01 DK 46057 (to I.J.F., R01 DK 67440 (to J.R.C.), and R01 DK 68216 (to J.R.C.), and a grant from Varian Biosynergy (to C.G.).

Address reprint requests to Chandan Guha, Albert Einstein College of Medicine, Montefiore Medical Center, Department of Radiation Oncology, 111 East 210 Street, Bronx, NY 10467. E-mail: cguhamd@pol.net; fax: 718-231-5064.

Copyright © 2008 by the American Association for the Study of Liver Diseases. Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hep.22573

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

Hepatocyte transplantation (HT) is of potential to be of major value in the treatment of inherited and acquired liver diseases.1,2 Because the host liver remains intact, HT places less metabolic burden on the engrafted tissue than does orthotopic liver transplantation. Although extensive preclinical studies and clinical trials have demonstrated the safety and feasibility of HT, its widespread clinical application has been hindered by several obstacles. Universal shortage of donor livers greatly limits the availability of high-quality hepatocytes for transplantation. Moreover, clinical experience has shown that the number of hepatocytes that can be engrafted safely during a single infusion is insufficient for completely curing liver-based inherited metabolic diseases.3 Therefore, several laboratories, including ours, have explored strategies to increase the efficiency of hepatocyte engraftment and to induce proliferation of the engrafted cells.4,6

After introduction of hepatocytes into the portal venous system by intrasplenic injection or portal vein infusion, the transplanted cells accumulate in periportal vessels and hepatic sinusoids.7,8 Most of these cells die,
probably from relative hypoxia and anoikis attributable to lack of cell adhesion,9 and phagocytosis by activated macrophages.7 A fraction of the hepatocytes cross the sinusoidal barrier, entering the space of Disse and integrating into liver cords by 7 days. Hepatic nonparenchymal cells are major modulators of initial hepatocyte engraftment. First, the hepatic sinusoidal endothelial cells (SEC) pose a physical barrier to the passage of the transplanted hepatocytes into the space of Disse, as suggested by the enhancement of hepatocyte engraftment by cyclophosphamide and doxorubicin, agents that disrupt hepatic SECs.8,10 Second, Kupffer cells and probably other inflammation-mediating cells may clear the transplanted cells, reducing the number of hepatocytes available for engraftment. Kupffer cell depletion and administration of anti-inflammatory agents have been reported to enhance hepatocyte engraftment modestly.7,11 Agents that disrupt the endothelial cells or inhibit Kupffer cell function do not, by themselves, induce subsequent proliferation of the transplanted cells. Because adult hepatocytes retain the capacity to undergo multiple rounds of cell division in response to mitotic stimuli and the liver-to-body-weight ratio is maintained within strict limits by robust physiological mechanisms,12,13 repopulation of the liver by transplanted hepatocytes can occur only if the transplanted cells proliferate at the expense of the host hepatocytes. Recent studies on the wing development of Drosophila melanogaster have shown that when two cell populations with different proliferative potential exist in the same compartment, growth of the rapidly proliferating cells leads to the death of the slower-growing cells, which would have survived in the absence of the more robustly proliferating cells.14,15 Similar mechanisms may exist in mammalian liver, so that if the engrafted hepatocytes possess a greater proliferative capacity than the host hepatocytes, the engrafted cells would grow preferentially in response to mitotic stimuli, progressively competing out the host hepatocytes.16 This has been achieved by preparatively treating rodent hosts with retorsine or lasiocarpine, which are pyrrolizidine alkaloids that induce a long-lasting cell cycle block, and 66% hepatectomy, which provides a strong mitotic stimulus to hepatocytes.17 Because of the carcinogenic potential of the pyrrolizidine alkaloids, we have pursued an alternative approach that consists of preparative hepatic irradiation (HIR), which reduces mitotic capacity of the host hepatocytes.18 The proliferative stimulus was provided by partial hepatectomy,18,19 portal vein ligation,20 and induction of apoptosis of host hepatocytes by expressing Fas ligand,21 or by expression of hepatocyte growth factor in the host liver.22,23 These preparative regimens led to the proliferation of the transplanted hepatocytes that engrafted initially as single cells, so that each engrafted cell gave rise to progressively enlarging clusters, which eventually replaced most of the host hepatocytes.

In the current study, we have focused on the effect of various doses of hepatic irradiation on the initial engraftment of hepatocytes. We hypothesized that HIR enhances initial integration of hepatocytes and their subsequent preferential proliferation by different mechanisms, so that the dose requirement for these two effects could be different. However, the rapidity and extent of eventual hepatic repopulation would depend on the total number of hepatocytes that are engrafted initially. Results of our rodent experiments demonstrate that, at low doses, HIR causes short-term disruption of hepatic SECs and inhibition of the phagocytic function of Kupffer cells, culminating in marked increase in the initial engraftment of transplanted hepatocytes. Furthermore, toward the development of clinical protocol of preparative HIR, we examined whether our findings were valid in nonhuman primates and demonstrated that at HIR doses as low as 10 Gy, there was a dramatic increase in the engraftment of donor porcine hepatocytes in cynomolgus monkeys, suggesting that augmentation of hepatocyte engraftment by low-dose HIR may be sufficient for the treatment of many inherited liver diseases, without the need for subsequent preferential proliferation of the engrafted cells.

Materials and Methods

Animals. Wild-type (Charles River Laboratories, NY) and dipeptidyl peptidase IV (DPPIV) deficient (Marion Bessin Liver Research Center) male F344 rats weighing 250 to 300 g were housed in the Institute for Animal Studies at the Albert Einstein College of Medicine. Male cynomolgus monkeys (5-9 kg body weight) were housed in the animal care facility at the University of Nebraska Medical Center. Female piglets, 6-8 weeks of age (8 kg to 15 kg), obtained from Larson Farms Inc. (Sargeant, MN), were kept in the animal care facility at the Mayo Clinic. The animal protocols were approved by the respective Institutional Animal Care and Use Committees.

HIR. HIR in rodents was performed as previously described.18 Briefly, DPPIV-deficient rats underwent laparotomy, followed by intraoperative irradiation of all hepatic lobes, except for the caudate lobe. HIR (0-50 Gy) was delivered using a Philips orthovoltage unit operating at 320 kVp, 5 mA, and 0.5 mm copper filtration at 200 cGy/minute after shielding of other abdominal organs with 2-mm-thick lead shields. Control DPPIV-deficient rats received a sham procedure, in which a laparotomy was performed but no HIR was delivered.
HIR (10 Gy) was delivered in anesthetized monkeys, using 6 MV photon energy beams after three-dimensional computerized tomographic treatment planning via a combination of coplanar and non-coplanar portals that were shaped by multi-leaf collimation to limit the dose to normal structures such as kidney and small bowel.

**Hepatocyte Isolation and Transplantation.** For rodent studies, donor hepatocytes were isolated from wild-type F344 rats with a modified collagenase perfusion method, as described before. Twenty-four hours after HIR and sham operation, \(1 \times 10^7\) hepatocytes (>80% viability) were injected intrasplenically in anesthetized animals. For primate studies, porcine hepatocytes were isolated, as previously described, and either transplanted immediately on isolation or within 24 hours of isolation after standard cold preservation.

**Rodent Studies.** DPPIV-deficient F344 rats received either HIR (5, 15, 30, and 50 Gy) or sham operation. To determine the effect of HIR on SECs, animals were sacrificed at various time points (2 hours, 6 hours, 1, 2, 3, and 7 days; \(n = 2-4\) for each time group), and blood was collected for serum chemistry, followed by perfusion of the liver with phosphate-buffered saline, and preparing liver tissues from each lobe for cryostat sections and paraffin embedding. Animals that received HT (\(n = 2\) for each time point) were sacrificed 1, 3, and 7 days after HT for histological examination of the transplanted hepatocytes in the liver.

**Detection of Apoptosis.** Terminal deoxynucleotidyl transferase–mediated 2′-deoxyuridine 5′-triphosphate nick end labeling (TUNEL) staining was performed using ApopTag in situ apoptosis detection kit (Chemicon International, Inc., Temecula, CA), according to the manufacturer’s instructions. TUNEL-positive apoptotic cells were counted in 20 high-power fields (HPF, \(\times 400\)) from each slide.

**Transmission Electron Microscopy.** Twenty-four hours after HIR, the rat liver was fixed by perfusion through the portal vein with 0.134 mol/L cacodylate buffer (pH 7.4), followed by 50 mL of a buffer containing 1.5% glutaraldehyde, 2% paraformaldehyde, 0.067 mol/L cacodylate, and 1% sucrose (pH 7.4). The left lobe was excised and cryopreserved in sucrose gradient (7.5% to 2.3%). Cryosections were postfixed in osmium tetroxide, stained en bloc with 1% uranyl acetate, dehydrated, and embedded in epoxy resin. Thirty consecutive sinusoidal areas in 50-nm liver sections were examined using a JEOL transmission electron microscope (Olympus, Tokyo, Japan).

**Serum Chemistry.** Serum levels of alanine aminotransferase (ALT) were measured in a clinical biochemical laboratory as markers of hepatocellular injury. Serum levels of hyaluronic acid (HA) were measured by the sandwich enzyme-binding assay (Corgenix Inc., Westminster, CO) as markers of SEC function.

**Kupffer Cell Assays.** Kupffer cells were visualized in 5-μm cryostat sections using mouse monoclonal ED2 anti-macrophage antibody by the indirect immunoperoxidase technique. In each slide, the number of Kupffer cells was counted in 20 consecutive periportal or pericentral areas in an HPF (\(\times 400\)). To determine the phagocytic activity of Kupffer cells, colloidal carbon clearance test was performed 24 hours after HIR or sham operation, according to protocols published before. Kupffer cells containing cytoplasmic carbon particles were counted in 20 consecutive periportal or pericentral areas under an HPF (\(\times 400\)) in each fresh-frozen liver section. Some animals received intravenous gadolinium chloride (GdCl\(_3\), 10 mg/kg body weight) to eliminate Kupffer cells.

**Detection of Transplanted Hepatocytes in Rodents.** To analyze survival and engraftment of DPPIV-positive transplanted cells in situ, 5-μm cryostat sections were stained for DPPIV histochemistry, and DPPIV enzyme activity was measured from liver homogenates, using previously published protocols. The sections were further stained for adenosine triphosphatase (ATPase) activity to colocalize bile canalicular ATPase and DPPIV activity in integrated donor hepatocytes, using published protocols.

In each liver section, the number of DPPIV-positive transplanted cells was counted in 10 low-power fields (LPF, 100×) and in 40 to 80 consecutive periportal areas in HPF (\(\times 400\)). The number of transplanted cell foci per 100 cm\(^2\) of liver was determined as described before.

**Porcine Albumin Enzyme-Linked Immunosorbent Assay.** The in vivo function of engrafted hepatocytes in primates was determined by assaying porcine albumin in recipient plasma every 2 to 3 days by a sandwich enzyme-linked immunosorbent assay (ELISA), using rabbit antipig albumin antibody as the capture antibodies (1:500; Accurate Chemical and Scientific Corp., Westbury, NY) and a horseradish peroxidase–conjugated goat anti-porcine albumin antibody (1:10,000; Bethyl Laboratories, Inc., Montgomery, TX) for colorimetric detection of porcine albumin.

**Statistical Analysis.** The results were expressed as mean ± standard deviation (SD). A statistical analysis of the data was made by the Mann-Whitney U test.

**Results**

HIR Induces Apoptosis and Dehiscence of Hepatic SECs. Because irradiation induces apoptosis in some cell types, such as lymphocytes, salivary gland acinar cells, and intestinal endothelial cells, we wanted to examine the ef-
Effect of HIR on hepatic SEC death. SEC apoptosis was determined by TUNEL staining after 50 Gy HIR, a dose that induced extensive liver repopulation by engrafted donor cells. TUNEL staining showed few apoptotic cells in normal liver (Fig. 1A). The number of apoptotic SECs increased significantly 6 hours after HIR (Fig. 1B, C, and F). In contrast, apoptotic hepatocytes were seen only occasionally (Fig. 1D). Twenty-four hours after HIR, the number of apoptotic cells decreased significantly (Fig. 1E), suggesting that the apoptotic cells had been cleared, probably by phagocytic cells, such as Kupffer cells.

Transmission electron microscopy of sham-irradiated liver demonstrated an intact lining of the normal hepatic sinusoidal endothelium with its characteristic fenestrae (Fig. 2A). In contrast, HIR induced nuclear crenellation and chromatin condensation (6-24 hours after HIR), followed by disruption of endothelial cell lining with exfoliation of endothelial cells from the space of Disse in irradiated livers (24-48 hours) (Fig. 2B).

HIR Increased Serum ALT and HA Levels Transiently. We determined serum ALT and HA levels, which are markers of hepatocellular and SEC injury, respectively (Fig. 2C). Serum ALT is released from dying hepatocytes. HA is cleared from the circulation by SECs by receptor-mediated endocytosis, whereby serum HA levels increase with SEC death. Serum ALT levels increased modestly (approximately 2-fold) after HIR, with peak levels at 6 hours (pre-HIR, 57.0 ± 13.7 U/L versus...
post-HIR, 138.7 ± 8.4 U/L; \( P < 0.05 \), eventually returning to baseline within 2 days. This indicated that the acute effect of HIR on hepatocellular death was minimal. In contrast, serum HA levels started increasing within 6 hours and peaked at 1 day after HIR (pre-HIR, 9.2 ± 1.3 ng/mL versus post-HIR, 175.5 ± 63.4 ng/mL, \( P < 0.01 \)). HA levels remained significantly elevated for 2 days (180.5 ± 51.4 ng/mL versus pre-HIR levels, \( P < 0.05 \)), and returned to pre-HIR values in 7 days. The transient elevation in HA levels suggested that the HIR-induced hepatic SEC damage was reversed in a week.

**HIR Reduced the Phagocytic Function of Kupffer Cells Without Affecting Their Numbers.** We next examined the effect of HIR on the number and phagocytic activity of intrahepatic resident macrophages, Kupffer cells. Liver sections were stained with ED2 (CD163), an anti-macrophage antibody, to estimate the number of Kupffer cells (Fig. 3A-D). Although a statistical significant difference was not achieved, Kupffer cells tended to be distributed more in perportal areas than in pericentral areas (pericentral, 19.5 ± 3.5; \( P = 0.127 \)) in control sham irradiated livers (Fig. 3A). HIR did not affect the number of Kupffer cells in either pericentral (Fig. 3B) or perportal areas (Fig. 3C).

As expected, administration of GdCl3 eliminated Kupffer cells almost completely from the liver (Fig. 3D). To evaluate the phagocytic function of Kupffer cells, India ink was injected intravenously and the clearance of colloidal carbon from the blood was measured. As demonstrated in Fig. 3J, the clearance of colloidal carbon was decreased after HIR. Because Kupffer cells primarily phagocytose carbon particles from the blood, the clear-
ance of colloidal carbon from the blood can be expressed as the phagocytic index of these cells. The phagocytic index (Fig 3K) of HIR-treated animals was significantly reduced than the sham irradiated controls (HIR, 0.012 ± 0.007 versus sham-irradiated, 0.024 ± 0.007, P < 0.05). Moreover, light microscopic examination of the liver after colloidal injection demonstrated that the number of peripoortal Kupffer cells that engulfed carbon particles in HIR-treated animals (Fig. 3F) was smaller than the sham-irradiated controls (HIR, 24.1 ± 3.8 versus sham-irradiated, 32.9 ± 3.3, P < 0.05) (Fig. 3E), indicating that HIR suppressed the phagocytic function of peripoortal Kupffer cells.

**HIR Augments Engraftment of Transplanted Hepatocytes in Rat Liver.** To examine whether SEC disruption and suppression of the phagocytic function of Kupffer cells by HIR enhances donor hepatocyte engraftment, DPPIV-deficient F344 rats received HIR at various doses (sham/0, 5, 15, and 50 Gy), followed in 1 day by transplantation of DPPIV-proficient F344 donor hepatocytes. The number of engrafted hepatocytes was determined by histochemical staining for DPPIV activity 1, 3, and 7 days after HT. In sham-irradiated livers, 1 to 2 donor hepatocytes were engrafted in 20% to 30% of peripoortal areas, 7 days after HT (Fig. 4A). The number of engrafted hepatocytes increased significantly with frequent clustering of donor cells in 70% to 80% of the peripoortal regions in rats receiving HIR. The efficiency of donor cell engraftment increased in a dose-dependent fashion (Sham 0 Gy, 45.7 ± 6.0 cells/cm² versus HIR 5 Gy, 219.3 ± 14.5 cells/cm², P = 0.0015, Fig. 4B-G). Thus, even at a 5-Gy dose, donor hepatocyte engraftment increased fourfold to fivefold. At the highest tested dose (50 Gy), there was a greater than 70-fold increase in the number of donor-derived hepatocytes (HIR 50 Gy, 3315 ± 37.5, P = 0.00009). Furthermore, in contrast to sham-irradiated livers, donor cells were frequently seen in clusters in irradiated livers by 7 days (Fig. 4D, E). Both...
the number and size of cell clusters increased with HIR dose, indicating that at higher doses HIR not only augmented the entry and engraftment of single or multiple adherent donor cells but also may have promoted selective donor cell proliferation (Fig. 4G).

To assess whether the engrafted hepatocytes integrated in the liver parenchyma, liver sections were co-stained with DPPIV and ATPase enzyme histochemistry. Figure 4F demonstrates characteristic linear co-staining with DPPIV and bile canalicular ATPase of integrated hepatocytes in the HIR-treated liver. Although most donor cells entered in hepatic sinusoids or liver plates of HIR-treated animals, these cells did not integrate in the parenchyma on day 1 post-HT (data not shown). However, by 7 days there was a significant increase in the number of integrated hepatocytes in HIR-treated animals, compared with sham controls.

**HIR Induces Preferential Proliferation of Engrafted Hepatocytes in Response to Hepatic Mitogenic Stimulus.** Finally, we wanted to examine whether the HIR-induced enhancement of donor cell engraftment results in a faster and preferential repopulation of the host liver by donor cells. To determine the liver repopulation capacity of engrafted hepatocytes, we provided a hepatic mitogenic stimulus by injecting a recombinant adenovirus expressing human hepatocyte growth factor intravenously, 7 days after HT, when engraftment and integration of donor cells in host liver parenchyma is complete. Three months after HT, liver repopulation was evaluated by DPPIV histochemical staining of the liver sections and by measuring DPPIV enzyme activity in liver homogenates. In animals treated with sham irradiation, donor cells failed to repopulate the liver. There was minimal proliferation of donor cells, as indicated by small clusters (2-4 hepatocytes) in animals receiving 5 Gy HIR, indicating that lower doses of HIR increase cell engraftment without affording a selective growth advantage to the donor cells. However, in animals receiving 15 and 50 Gy HIR, donor cells preferentially proliferated resulting in extensive repopulation of the host liver (Fig. 5A). Compared with sham-irradiated livers, there was a significant increase in DPPIV enzyme activity in animals receiving HIR with a gradual dose response (Fig. 5B), indicating that higher doses of HIR enabled preferential proliferation of the engrafted hepatocytes.

**HIR Augmented Engraftment of Porcine Hepatocytes in Cynomolgus Monkeys.** Encouraged by our findings that low-dose HIR enhanced hepatocyte engraftment in rats, we wanted to develop a clinically feasible protocol of preparative HIR for HT. To validate the HIR effect in nonhuman primates, in preliminary studies we infused porcine hepatocytes into the portal vein of cynomolgus monkeys after right portal vein embolization (PVE), low-dose HIR (10 Gy), or a combination of HIR and right PVE. PVE was performed to stimulate compensatory hypertrophy of the unoccluded lobes, because portal vein branch occlusion was shown to induce atrophy of occluded hepatic lobes, thereby providing mitotic stimulation. All animals received an identical immune suppression regimen, which included Thymoglobulin, methylprednisolone, and anti-CD25 at the time of HT, and cyclosporine A, FTY720 (Fingolimod), RAD (Everolimus), and methylprednisolone, thereafter. The in vivo function of transplanted hepatocytes was determined by measuring serum porcine albumin, which was used as a surrogate marker for initial engraftment of donor hepato-
tocytes. Porcine albumin levels in monkeys treated with PVE increased only slightly after HT in a pattern identical with that seen following transplantation in naïve monkeys (data not shown), indicating that PVE does not, by itself, enhance initial engraftment of donor hepatocytes (Fig. 6). Preparative HIR, followed by transplantation of porcine hepatocytes, 1 day post-HIR, resulted in a large increase in porcine albumin levels even when the transplanted cells were preserved in cold storage before transplantation. PVE and HIR resulted in a 30-fold increase in porcine albumin levels after HT using freshly isolated hepatocytes, confirming the extent to which preparative HIR augments initial engraftment of donor hepatocytes in monkeys, and indicating that engraftment may be further amplified by selective proliferation of engrafted cells induced by PVE.

Discussion

Our results show that, at low doses, preparative HIR of recipient rats enhances the overall efficiency of donor hepatocyte engraftment. HIR disrupted the hepatic sinusoidal endothelial barrier and suppressed the phagocytic function of Kupffer cells transiently, which was, nevertheless, sufficient for facilitating the engraftment of transplanted hepatocytes and integration into liver cords. Enhancement of initial engraftment occurred at low HIR doses (5-10 Gy), whereas higher doses were required for subsequent proliferation of the individual engrafted cells, supporting our hypothesis that the mechanisms of these two effects were different.

Induction of apoptosis of hepatic SECs by low doses of HIR could have facilitated the entry of the donor hepatocytes into the perisinusoidal space of Disse and their migration into the liver plate. Similar effects were reported using agents that induce SEC damage, such as cyclophosphamide or doxorubicin. However, there are some important differences with HIR. First, drugs act on the whole liver, whereas the effect of HIR can be spatially confined to parts of the liver, thereby augmenting engraftment only in the irradiated liver lobes. Instead of using an agent that induces sinusoidal cell injury in the whole liver, partial liver irradiation increases the safety of the preparative regimen by leaving portions of the normal liver intact. Second, drugs have systemic toxicity, such as bone marrow toxicity, whereas local liver irradiation fails to exhibit systemic toxicity. Finally, agents that augment engraftment usually do not induce proliferation of hepatocytes. At lower doses, up to 10 Gy, HIR augments donor cell engraftment. At higher doses, in addition to enhancing engraftment, HIR also reduces the proliferative capacity of host hepatocytes, thereby providing a competitive proliferative advantage to the engrafted donor cells. Therefore, the two effects of HIR should act synergistically to result in rapid and massive repopulation of the liver, making HIR highly desirable as a preparative regimen for HT.

Although the fenestrated hepatic sinusoidal endothelium allows regulated access of macromolecules to the space of Disse, it is an important physical barrier to the engraftment of transplanted hepatocytes. HIR induced apoptosis of SEC within 6 hours, as demonstrated by TUNEL staining. This effect was comparable to that seen in the small intestinal microvascular endothelium, which underwent apoptosis within 4 hours of whole body irradiation in C57Bl/6 mice. Transmission electron microscopy of rat livers demonstrated that the disruption of the hepatic SECs was first evident by 6 hours after HIR and peaked in 24 to 48 hours. We therefore transplanted hepatocytes 1 to 2 days after HIR. We also determined serum HA levels as a marker of hepatic SEC integrity. HA is a straight-chain glycosaminoglycan polymer, present in the extracellular matrix. More than 90% of circulating HA is removed by hepatic SECs, via specific HA receptor-mediated endocytosis. Increasing levels of circulating HA serves as a biomarker of dysfunction of hepatic SEC after liver injury. We found that serum HA levels reached peak values within 24 hours of HIR, and HA...
returned to baseline within 3 to 7 days of HIR, probably because of the restoration of the sinusoidal endothelial barrier by endothelial cell proliferation. It is possible reduced clearance by SECs was compensated over time by enhanced renal excretion, which is the second major mechanism of removal of circulating HA.  

As the largest population of resident macrophages in the body, Kupffer cells are also reported to play an important role in eliminating transplanted cells shortly after hepatocyte transplantation. In contrast to the effect of HIR on SECs, we found that the number of Kupffer cells was not affected by HIR. However, HIR suppressed the phagocytic function of the Kupffer cells, resulting in a decrease in colloidal carbon clearance. HIR-induced suppression of the phagocytic activity of Kupffer cells could contribute to improved survival of the transplanted hepatocytes by allowing them more time to engraft and integrate into the hepatic architecture. Because HIR can disrupt endothelial cells and inhibit Kupffer cell phagocytosis at the same time, it is difficult to separate the effect of the two events. However, previous studies have demonstrated that elimination of Kupffer cells with systemic administration of gadolinium chloride can, at best, augment hepatocyte engraftment by twofold to fourfold. In contrast, HIR can improve hepatocyte engraftment by fivefold to 70-fold in a dose-dependent manner, suggesting that, in addition to its effect on Kupffer cell function, HIR enhances engraftment by additional mechanisms, such as disruption of hepatic sinusoidal endothelium.

Our results demonstrate that at HIR doses as low as 5 Gy, there is significant enhancement of engraftment of the donor hepatocytes in rat liver. Although our rodent studies have demonstrated convincingly the role of HIR in augmenting engraftment and subsequent proliferation of donor hepatocytes, primate studies represent an absolutely necessary step toward the application of preparative hepatic irradiation in humans. This is particularly true because the irradiation effects in the liver vary in different classes of species. Our results demonstrate that, as in the case of rodents, a single dose of 10 Gy preparative HIR was sufficient to increase significantly the engraftment of donor porcine hepatocytes in cynomolgus monkeys, as shown by the secretion of porcine albumin in the plasma. Additionally, immunosuppressive effects of the local irradiation could contribute to the improved survival of allogeneic hepatocytes or porcine xenograft. In clinical situations, radiation-induced liver damage occurs after irradiation of the whole liver at doses of 35 Gy or higher. Thus, the ability of low doses of HIR to increase the engraftment and integration of transplanted hepatocytes in rodents and in pilot studies in monkeys is highly significant in terms of potential translation of this strategy to clinical application. Furthermore, the technical feasibility of delivering HIR to isolated regions of the liver, without inducing radiation-induced liver disease, makes HIR an attractive preparative regimen for HT-based treatment of inherited liver diseases.

References


