Amelioration of Radiation-induced Liver Damage in Partially Hepatectomized Rats by Hepatocyte Transplantation

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Abstract

Hepatic tumors often recur in the liver after surgical resection. Postoperative radiotherapy (RT) could improve survival, but curative RT may induce delayed life-threatening radiation-induced liver damage. Because RT inhibits liver regeneration, we hypothesized that unirradiated, transplanted hepatocytes would proliferate preferentially in a partially resected and irradiated liver, providing metabolic support. We subjected F344 rats to hepatic RT and partial heptectomy with or without a single intrasplenic, syngeneic hepatocyte transplantation. Hepatocyte transplantation alleviated radiation-induced liver damage and improved survival of rats receiving RT after partial heptectomy. We further demonstrated that transplanted hepatocytes extensively repopulate and function in a heavily irradiated rat liver.

Introduction

Novel therapeutic strategies are needed to decrease mortalities in patients with liver cancer. Despite apparently complete surgical resection, liver cancer frequently recurs (1). In many carcinomas, such as head and neck, uterus, and breast carcinoma, the combination of surgery and RT has been successful in improving the outcome. However, the major limitation in applying such a strategy to liver cancers is the induction of potentially lethal RILD, which may develop when more than 30–35 Gy of radiation are administered to the whole liver (2, 3). Moreover, the partially resected liver may be more susceptible to RILD because radiation is known to impair liver regeneration in this setting (4). HT has been shown to decrease the mortality in experimentally induced liver failure (5). HT into the liver also ameliorates pyrrolizidine alkaloid-induced liver disease (6), which is associated with veno-occlusive disease (7), similar to that seen in RILD (2). We hypothesized that radiation of partially resected liver will cause hepatocyte injury and suppress liver regeneration, during which transplanted hepatocytes with a normal regenerative potential would proliferate and repopulate the liver. The metabolic support provided by transplanted hepatocytes should ameliorate consequences and mortality associated with RILD. To test this hypothesis, rats were subjected to 68% heptectomy followed by intraoperative radiation to the residual liver. Hepatocytes isolated from syngeneic donors were then transplanted into the liver by intrasplenic injection, which results in the translocation of a major fraction of the transplanted hepatocytes to the liver with permanent engraftment and function (8, 9). The results indicated that HT ameliorated RILD and significantly improved survival. In addition, there was extensive repopulation of the liver by the transplanted hepatocytes in animals receiving RT + PH.

Materials and Methods

Animals. Male F344 rats weighing 250–300 g were obtained commercially (Charles River Laboratories, NY) and housed in the Institute for Animal Studies at the Albert Einstein College of Medicine. The DPP IV-deficient (DPP-IV−/−) F344 rats were provided by the Special Animals Core of the Marion Bessin Liver Research Center. The animals were provided with pelleted chow and water ad libitum and were kept under 14-h light/10-h dark cycles. The animal protocols were approved by the institutional Animal Care and Use Committee.

Experimental Design. For survival experiments, 34 male rats were randomly assigned to two experimental groups (Table 1). Rats in one group (n = 17) underwent 68% PH followed immediately by intraoperative whole liver RT with 50 Gy. The dose was selected on the basis of a preliminary radiation dose escalation (15–50 Gy) experiment, which showed that a single 50-Gy RT dose to the liver after PH produced severe RILD and high mortality in F344 rats. Rats in a second group (n = 17) underwent 68% PH + RT as described above and subjected to HT 4 days later. For comparison, five rats received RT without PH, and five rats underwent PH without RT. To identify transplanted cells in the liver of recipients, DPP IV−/− F344 rats were used. DPP IV−/− rats underwent HT after PH + RT and were sacrificed at 12 weeks. For comparison, two additional groups of three DPP IV−/− rats each received HT with or without PH, without any liver RT. Animals from each of these groups were killed at 12 weeks for histological examination of the liver.

Surgical Procedures. Anesthesia was induced by isofluorane inhalation using a closed circuit. The abdomen was opened by a midline incision, and whole liver RT with 50 Gy. The dose was selected on the basis of a preliminary radiation dose escalation (15–50 Gy) experiment, which showed that a single 50-Gy RT dose to the liver after PH produced severe RILD and high mortality in F344 rats. Rats in a second group (n = 17) underwent 68% PH + RT as described above and subjected to HT 4 days later. For comparison, five rats received RT without PH, and five rats underwent PH without RT. To identify transplanted cells in the liver of recipients, DPP IV−/− F344 rats were used. DPP IV−/− rats underwent HT after PH + RT and were sacrificed at 12 weeks. For comparison, two additional groups of three DPP IV−/− rats each received HT with or without PH, without any liver RT. Animals from each of these groups were killed at 12 weeks for histological examination of the liver.

Whole Liver Radiation. Immediately after PH, animals were positioned on a specially constructed polystyrene (aquadest) platform. A jig was aligned on aquaplast and separated into two compartments through which a longitudinal port (5.0 × 7.0 cm) is accessible for irradiation. Two 3 × 4-cm lead shields (2 mm thick) were wedged beneath the liver and overlying the stomach and intestines without compressing the hepatic and aortic vessels. A 320 MGC Philips orthovoltage unit operating at 320 kVP, 10 mA, and 0.5 mm copper filtration was used (320 cGy/min hepatic exposure to the midline at a 2-cm depth within the jig at a 35 cm-source-to-surface distance). Thermoluminescence dosimetry was used for a liver phantom within the jig as the basis for all corrected dose calculations. After irradiation, animals were examined daily. Rats with severe cachexia or moribund condition were euthanized and recorded as nonsurvivors.

Hepatocyte Isolation and Perfusion. Cells were isolated with a modified collagenase perfusion method using male F344 rats, as originally described by Berry and Friend (11). After liver dissection, cells were filtered through an

Received 7/20/99; accepted 10/15/99.

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Supported in part by NIH Grants ROI DK-46957 (to J. R-C.), ROI DK-59137 (to N.R.C.), ROI DK-46952 (to S. G.), and DK-P30–41296 to Marion Bessin Liver Research Center (David A. Shafritz, M.D., PI) and by grants from the department of Radiation Oncology, Montefiore Medical Center (Bronx, NY).

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1 The abbreviations used are: RT, radiotherapy; PH, partial heptectomy; HT, hepatocyte transplantation; RILD, radiation-induced liver damage; DPP IV, dipeptidyl peptidase IV.
Results and Discussion

Intraoperative RT has been used to improve local control for a variety of gastrointestinal cancers and could be used to deliver a high dose of radiation to the tumor resection bed and adjacent liver tissues harboring micrometastasis with shielding of other abdominal organs. Unfortunately, in therapeutically effective doses, hepatic irradiation causes severe RILD, often resulting in death after 3–4 months. The purpose of this study was to begin to examine whether HT could modify this process. Because RILD usually manifests itself within 4 months after irradiation, we observed irradiated animals for at least 120 days or until they became moribund.

Survival. During the 120 days of observation, none of the rats subjected to PH alone or to RT alone died. Seventy percent of rats (12 of 17) receiving RT + PH died in 12 weeks. In the RT + PH + HT group, the mortality was reduced to 35% (6 of 17; Fig. 1). The median survival in the RT + PH group was 65 days (8 of 17 died during the first 6 weeks) and >120 days ($P = 0.02$) in the RT + PH + HT group (only 1 of 17 rats died during the first 6 weeks).

Histopathological Changes. Examination of paraffin sections of formalin-fixed liver biopsy specimens showed minimal changes in the animals subjected to RT without PH, including occasional foci of inflammatory cells and mild microvesicular steatosis. In animals that received RT + PH and died within the first 6 weeks, severe histopathological changes of RILD, including extensive loss of hepatocytes (Fig. 2A), and various degrees of micro- and macrovesicular steatosis in centrilobular areas (Fig. 2B) were seen. The predominantly centrilobular steatosis was reminiscent of other forms of liver injury, including oxidative stress as in alcoholic or toxic hepatitis (14) and the perivenous hepatocellular loss that has been reported in humans (2). In animals in the PH + RT group that survived beyond 12 weeks, the steatosis was less pronounced, but focal hepatic necrosis and various degrees of portal fibrosis and bile duct proliferation were seen (Fig. 2, D and E). Hepatocytes with multiple nuclei, nuclear pleomorphism, and megalonuclei were observed. These findings indicated the accumulation of postmitotic hepatocytes (15), suggesting a radiation-induced cell cycle block.

In the PH + RT + HT group, only 1 animal died during the first
6 weeks. Therefore, to examine histological changes at early time points, six additional rats were subjected to PH + RT, of which 3 were sacrificed 1 and 3 weeks later, respectively. Hepatic histopathological changes were much less prominent (Fig. 2C) in this group than in rats undergoing PH + RT. There was no steatosis, although two rats showed minimal loss of centrizonal hepatocytes.

Transplant recipients in the PH + RT + HT group, which survived more than 12 weeks, did not develop the late histopathological changes characteristic of RILD, such as bile duct proliferation and fibrosis (Fig. 2F). In contrast, livers of rats in the PH + RT + HT group that died after 7 weeks manifest features of RILD including centrizonal steatosis, cell loss, and bile duct proliferation.

The extensive bile duct proliferation seen in animals undergoing PH + RT without HT may have arisen from preexisting bile duct epithelium or may represent the proliferation and differentiation of putative periportal stem cells. After PH, the lost liver mass is normally replaced by the proliferation of mature hepatocytes, but when the mature hepatocytes lose their proliferative capacity, such as after 2-acetylaminofluorane treatment (16), proliferation of periportal stem cells comes into play. Radiation could induce cell cycle block and thereby inhibit hepatocyte regeneration. The increased cell loss seen in RILD over a prolonged time period could be the result of the postmitotic cell death of injured hepatocytes. It is possible that the failure of the irradiated hepatocytes to undergo compensatory regeneration results in the expansion of the periportal stem cells, leading to bile duct proliferation. With continuing hepatocellular loss in animals receiving PH + RT, proliferative stimuli remain in the host liver, which allows nonirradiated transplanted hepatocytes to eventually proliferate and extensively repopulate the host liver. In long-term survivors after HT, the lost liver mass is replenished by proliferation of the transplanted hepatocytes, which may have prevented the compensatory expansion of periportal stem cells and consequent bile duct proliferation. In contrast, the presence of bile duct proliferation in animals that died after receiving PH + RT and HT could be due to failure of engraftment and/or repopulation of the transplanted hepatocytes in these animals. Repeated HT has been shown to enhance liver repopulation (17) and might further improve the survival rate in irradiated rats.

**Engraftment and Liver Repopulation by the Transplanted Cells.** For histochemical identification of the engrafted hepatocytes within the host liver, hepatocytes obtained from normal F344 rats were transplanted into congeneric DPP IV− F344 recipients (12). DPP IV staining of cryostat sections of DPP IV− host livers will only stain transplanted DPP IV+ hepatocytes (red staining of plasma membranes). Liver sections from recipients subjected to PH without RT showed only isolated donor cells scattered within the liver cords even after 12 weeks (Fig. 3, A and B). In contrast, the RT + PH + HT group, by 12 weeks, there was a massive proliferation of the donor hepatocytes, repopulating most of the liver (Fig. 3, C and D). For biochemical evaluation of the extent of liver repopulation by
the DPP IV+ donor hepatocytes, DPP IV activity was assayed in liver homogenates. Untreated DPP IV− rat livers showed no DPP IV activity. The fraction of the hepatocyte mass replaced by the progeny homogenates. Untreated DPP IV rat livers showed no DPP IV activity. 1

In conclusion, we demonstrate that the combination of PH and whole liver irradiation leads to extensive RILD in rats, which should allow systematic analysis of mechanisms involved in RILD. Furthermore, we demonstrate that HT has the potential to ameliorate the consequences of RILD with improved survival of animals. The capability of transplanted hepatocytes to divide repeatedly and repopulate the host liver has been shown in inherited disorders that cause the death of mature hepatocytes, such as in AlbuPA transgenic mice (18), in Long Evans Cinnamon rats that have hepatocellular copper overload (19), and in fumarylacetoacetate hydrolase-deficient mice (20). In this study, we show that such massive proliferation and repopulation of the liver can also occur in RILD after PH + RT. Our rat model exhibits many characteristic features of human RILD, including centrilobular hepatocellular injury and atrophy. However, events associated with veno-occlusive disease, such as the formation of platelet lakes and the fibrin deposits that are seen in human RILD, were not found in this model. The ability of transplanted hepatocytes to proliferate, repopulate, and function in a heavily irradiated rat liver warrants investigation into whether such a strategy could ameliorate RILD and benefit patients requiring hepatocellular resection and adjutant RT for intrahepatic malignancies. Whether targeted ablation of the liver with radiation could help increase liver repopulation with hepatocyte transplantation in noncancer settings is another possibility worth considering.

References
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