Liver Regeneration and Bioenergetic Changes Following Hepatic Irradiation and Hepatocyte Transplant *in Vivo* by ^{31}P MRSI

C. Landis[1], L. Liu[2], B. Xi[2], J. Roy-Chowdhury[1], H. Hetherington[3], C. Guha[2]

[1]Medicine, Albert Einstein College of Medicine, Bronx, New York, United States, [2]Radiation Oncology, Albert Einstein College of Medicine, Bronx, New York, United States, [3]Radiology, Albert Einstein College of Medicine, Bronx, New York, United States

Introduction: Radiation treatment is often used as adjuvant therapy following the surgical resection of many cancers. However, it has limited usage in the treatment of primary or metastatic liver cancers due the development of radiation induced liver damage (RILD). Work in our laboratory has demonstrated that hepatocyte transplant (HT) could ameliorate RILD in rats (1). Unfortunately there are few predictive markers for RILD and clinical symptoms are typically revealed in late stages. Hence, a noninvasive method of monitoring the success of HT in ameliorating RILD is desirable for clinical application. Multiple studies have shown that ^{31}P MRS can reliably monitor liver regeneration and energetic status following partial hepatectomy (PH) in animals (2) and humans (3). PH and whole liver irradiation (PHRT) is an established rodent model of RILD and can be used as a preparative regimen for hepatocyte transplant (1). To study the metabolic changes that occur in RILD with and without HT, ^{31}P MRSI data was acquired from rats treated with PH alone, PHRT or PHRT with HT.

Methods: Fisher 344 rats were subjected to PH alone (n=7), PHRT (n=14), or PHRT-HT (n=10). PHRT treated animals received a single dose of 50 Gy localized to the liver immediately following a two-third PH. In the PHRT-HT group, 24hr after PHRT, rats were transplanted with $2-4\times10^6$ hepatocytes via the spleen. Donor hepatocytes were isolated from syngeneic donor livers using a standard 2-step perfusion technique. The freshly isolated hepatocytes were also transfected with the green fluorescent protein (GFP) gene under the liver specific albumin promoter by incubating with a FLAP-lentiviral-GFP vector for 3 hours at 37° prior to HT. The FLAP-lentiviral vector was recently described (4) and allows for rapid and efficient transfection of cell suspensions. Expression of GFP by donor hepatocytes allowed for subsequent verification of hepatocyte engraftment and repopulation by immunohistochemistry.

^{1}H MRI and ^{31}P MRSI data were acquired with a 9.4 T Varian INOVA MR system using an 8cm i.d. [1]H linear birdcage coil and a 2.5 cm ^{31}P surface coil. Rats were intubated and anaesthetized with 1.5-2% Isoflurane. The core body temperature was maintained at 36-37° during each study. For ^{31}P MRSI, a 90° adiabatic hyperbolic secant excitation pulse and 3D spherical k-space sampling scheme on a $13\times13\times13$ grid was used (FOV=48x48x48mm, TR=1s). MRS studies were performed 1 day and 1, 2 (PH, PHRT only), 4, and 6 weeks after PH, PHRT, or PHRT-HT (n=3-4 rats/time point). For analysis, ^{31}P MRSI voxels from the same region (six contiguous voxels) were selected from each study (ROI in Figure 1). [31]P metabolite concentrations were estimated using an external standard and *in vivo* T_1 measurements to correct for partial saturation effects.

Figure 1.

Results and Discussion: An MR image and localized ^{31}P MRS spectrum from a rat 30 days after PHRT-HT is displayed in Figure 1. The time course of estimated [γ-ATP] for each group is displayed in Fig. 2a. The [γ-ATP] in the PHRT and PHRT-HT groups remains depressed throughout the study, but returns to normal following PH within 2 weeks. Although [γ-ATP] of the PHRT-HT group is increased compared to the PHRT group at 6 weeks (p<0.05), they are depressed relative to the PH group (p<0.005). This is not surprising, given that less than 10% repopulation of donor hepatocytes was observed by GFP immunohistochemisty in PHRT-HT rats sacrificed at 6 weeks, consistent with previous studies (1). The time course for the ratio of [ATP]/[Pi] is shown in Fig. 2b. Neither the PHRT nor the PHRT-HT rats return to control ATP/Pi values at 45 days (p<0.02, compared to PH only). It should be noted that the effects seen in Figure 2a and b for the PHRT group represent a selected population of rats that survived to a given time point for the MRS study. The percent survival curve for all three groups is shown in Figure 2c. This suggests that there may be threshold [γ-ATP] that predicts mortality in RILD and possibly other acute and chronic liver diseases.

Conclusions: ^{31}P MRSI has been used to monitor the evolution of RILD following PHRT. This study suggests that ^{31}P MRS estimated [γ-ATP] reflects bioenergetic reserve while the ATP/Pi ratio reflects, in part, proliferation of host and donor liver cells. Since, RT inhibits hepatic regeneration following PH (1), reduced [γ-ATP] in the PHRT group suggests RT-induced hepatic metabolic injury. The persistently reduced ATP/Pi ratio in the PHRT-HT group coupled with normalizing [γ-ATP] indicates that transplanted hepatocytes can simultaneously have restorative metabolic function and proliferate over time to replace host hepatocytes damaged by RT. Given the spatial heterogeneity of RILD and HT repopulation, MR based methods may prove to be better suited to monitor RILD and HT than approaches that include serial biopsy.

Figure 2.

References: 1. Guha et al. Cancer Res. 1999 Dec 1;59(23):5871-4 **2.** Corbin et al. Hepatology. 2002 Aug;36(2):345-53. **3.** Zakian et al. Magn Reson Med. 2005 Aug;54(2):264-71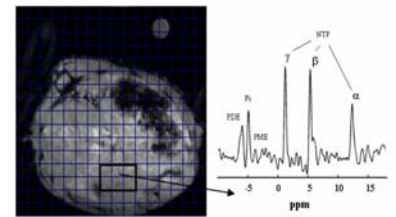. **4.** Giannini et al. Hepatology. 2003 Jul;38(1):114-22.