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Small animal magnetic resonance imaging: an efficient tool to assess liver volume and intrahepatic vascular anatomy

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ABSTRACT

Background: To develop a noninvasive technique to assess liver volumetry and intrahepatic portal vein anatomy in a mouse model of liver regeneration.

Materials and methods: Fifty-two C57BL/6 male mice underwent magnetic resonance imaging (MRI) of the liver using a 4.7 T small animal MRI system after no treatment, 70% partial hepatectomy (PH), or selective portal vein embolization. The protocol consisted of the following sequences: three-dimensional–encoded spoiled gradient-echo sequence (repetition time per echo time 15 per 2.7 ms, flip angle 20°) for volumetry, and two-dimensional –encoded time-of-flight angiography sequence (repetition time per echo time 18 per 6.4 ms, flip angle 80°) for vessel visualization. Liver volume and portal vein segmentation was performed using a dedicated postprocessing software. In animals with portal vein embolization, portography served as reference standard. True liver volume was measured after sacrificing the animals. Measurements were carried out by two independent observers with subsequent analysis by the Cohen κ-test for interobserver agreement.

Results: MRI liver volumetry highly correlated with the true liver volume measurement using a conventional method in both the untreated liver and the liver remnant after 70% PH with a high interobserver correlation coefficient of 0.94 (95% confidence interval, 0.80–0.98 for untreated liver [$P < 0.001$] and 0.90–0.97 after 70% PH [$P < 0.001$]). The diagnostic accuracy of magnetic resonance angiography for the occlusion of one branch of the portal vein was 0.95 (95% confidence interval, 0.84–1). The level of agreement between the two observers for the description of intrahepatic vascular anatomy was excellent (Cohen κ value = 0.925).

Conclusions: This protocol may be used for noninvasive liver volumetry and visualization of portal vein anatomy in mice. It will serve the dynamic study of new strategies to enhance liver regeneration *in vivo*.

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1. Introduction

The assessment of liver volume and perfusion is paramount in many clinical situations. It is extensively used in the preoperative setting before major liver resection and living donor liver transplantation to measure the future remnant liver (FRL) and prevent liver insufficiency. Indeed, postoperative insufficient functional liver mass typically leads to death of the patients within a few days [1]. To overcome this issue, one protective strategy is to occlude a main branch of the portal vein before surgery to induce hypertrophy of the FRL [2–7]. This requires optimal monitoring of the changes in liver volume and a complete picture of the intrahepatic vascular anatomy.

A number of animal models have been developed to study liver regeneration, including partial hepatectomy (PH). Up to now, the intrahepatic vascular anatomy is studied by repetitive x-ray imaging of animals after intravenous contrast injection (i.e., portography), which is an invasive procedure [4], technically demanding, and hence seldom used. Because liver regeneration is a dynamic process, important parameters might be lost or altered, if analyzed after having sacrificed animals. Moreover, a large number of animals are usually required for such experiments. Another method is the contrast-enhanced microscopic computed tomography (microCT) [8,9]. However, this method is limited by the need of contrast injection to differentiate soft tissues and body fluids [8].

Magnetic resonance imaging (MRI) is claimed to be a good alternative compared with a conventional method (i.e., measurements of volume *ex vivo* after sacrificing animals) to assess organ and tumor volumes in animal models [10,11]. However, data on mouse liver are scarce and there is lack of standardization [12–14]. Currently, new advances in imaging techniques provide unique possibilities to visualize the internal structure of organs and to collect systematic imaging data. New open source software, such as OsiriX (Pixmeo, Geneva, Switzerland), was developed to analyze imaging resources and give an accurate visual representation of these data [15]. The use of such software associated with the small animal MRI may contribute to the development of new methods for morphologic analysis of organs in mice and improve the tools available to study potential proregenerative agents in the liver. In addition, using MRI, animals can act as their own controls in repetitive measurements. Finally, a significant reduction in the number of animals used for experimentation would be achieved, and a decrease in interference with animal well-being and physiology status related to surgery required for conventional methods.

This study aimed at exploring the efficacy of small animal MRI to assess liver hypertrophy during liver regeneration after PH and modifications of intrahepatic vascular anatomy after portal vein occlusion in mice.

2. Materials and methods

2.1. Animals

All experiments were performed in 8–12-wk-old C57BL/6 male mice (Harlan, Horst, The Netherlands). Animals were housed in the animal facility of the University Hospital of Zurich with unrestricted access to standard chow and water. All experiments were approved by the Veterinary Office of Zurich and were performed in accordance with the institutional animal care guidelines.

2.2. Surgical procedures and anesthesia

All surgical procedures were performed between 8 and 12 h. All interventions were performed under constant isoflurane inhalation for surgery and MRI. For portography, an intraperitoneal bolus injection of pentobarbital (5 mg/kg body weight) was used as anesthesia to facilitate the transfer of animals from the operating room to imaging facilities. As analgesic, buprenorphine (0.1 mg/kg body weight) was applied subcutaneously and repeated 8–12 h later if required. After surgery, all animals were allowed to recover on a heating pad.

Seventy percent PH consisted of removal of the middle and left liver lobes with standard microsurgical techniques as described previously with some adaptations [16]. Briefly, the abdomen was opened by a midline incision. The left and middle lobes were ligated with a silk thread and resected. The gallbladder was also removed after ligation of the cystic duct. Finally, the abdomen was closed with a silk running suture. Sham surgery consisted of opening the abdominal cavity and liver mobilization without lobe resection.

Portal vein embolization (PVE) and portography procedures started with an abdominal midline incision. The peritoneal cavity was opened and the liver was freed from its ligaments. For portography, a puncture of the central portal vein was performed with a 29-gauge insulin needle (BD, Allschwil, Basel, Switzerland) attached to a 1-mL syringe connected to a butterfly tube . Vascular opacification (Fig. 1A) was performed with Sodium and Meglumine Ioxitalamate contrast agent (1:4 diluted with 0.9% NaCl: 0.25 mL Sodium and Meglumine Ioxitalamate + 0.75 mL NaCl 0.9%). At the end of the portography, the needle was removed from the central portal vein and hemostasis was achieved by the pressure on the puncture point. Seventy percent PVE was performed after blocking the portal branches to the caudate and right lobes with vascular clamps (Aesculap; Ref FE710K, B Braun, Melsungen AG, Germany). A puncture of the central portal vein was then performed with a 29-gauge insulin needle (BD) attached to a 1-mL syringe filled with 10 μL of microspheres made from trisacryl cross linked with gelatin (Embosphere, Biosphere Medical SA, Rockland, MA). Portography was then performed to confirm the occlusion of portal vessels (Fig. 1B).

2.3. Magnetic resonance imaging

Anesthetized mice were fixed to a warming pad in the ventral position. MRI was performed with a 4.7 T small animal magnetic resonance imager (Pharmascan; Bruker Biospin, Ettlingen, Germany) using a linearly polarized birdcage whole-body mouse coil. The protocol consisted after gradient-echo localizers in all three directions of the following sequences: a three-dimensional (3D)–encoded spoiled gradient-echo sequence with repetition time 15 ms, echo time 2.7 ms, flip angle 20°, field-of-view 24 × 30 × 30 mm^3, matrix size 256 × 256 × 128, resolution 0.09 × 0.117 × 0.234 mm^3, 7