Imaging evaluation of the liver using multi-detector row computed tomography in micropigs as potential living liver donors

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The shortage of organ donors has stimulated interest in the possibility of using animal organs for transplantation into humans. In addition, pigs are now considered to be the most likely source animals for human xenotransplantation because of their advantages over non-human primates. However, the appropriate standard values for estimations of the liver of micropigs have not been established. The determination of standard values for the micropig liver using multi-detector row computed tomography (MDCT) would help to select a suitable donor for an individual patient, determine the condition of the liver of the micropigs and help predict patient prognosis. Therefore, we determined the standard values for the livers of micropigs using MDCT. The liver parenchyma showed homogenous enhancement and had no space-occupying lesions. The total and right lobe volumes of the liver were 698.57 ± 47.81 ml and 420.14 ± 26.70 ml, which are 51.74% and 49.35% of the human liver volume, respectively. In micropigs, the percentage of liver volume to body weight was approximately 2.05%. The diameters of the common hepatic artery and proper hepatic artery were 6.24 ± 0.20 mm and 4.68 ± 0.13 mm, respectively. The hepatic vascular system of the micropigs was similar to that of humans, except for the variation in the length of the proper hepatic artery. In addition, the diameter of the portal vein was 11.27 ± 0.38 mm. In conclusion, imaging evaluation using the MDCT was a reliable method for liver evaluation and its vascular anatomy for xenotransplantation using micropigs.

Keywords: liver evaluation, micropig, multi-detector row computed tomography, xenotransplantation

Introduction

Transplantation often used to treat severe organ failure. Thus, liver transplantation has been suggested as an ultimate choice to treat end-stage liver disease [4,5]. The growing clinical indications and advances in medical technologies for liver transplantation have led to an expansion of transplantation procedures. As a reflection of the severe shortage of cadaveric organ donation, living donor liver transplantation has been more frequently considered in recent years [5]. Despite of this effort to ameliorate the shortage of liver donation, there remains an organ crisis due to a demand and supply imbalance with many more patients requiring liver transplants than there are organs available for the procedure [26]. Out of the need to expand the donor pool and alleviate this critical organ shortage, the concept of animal-to-human transplantation (xenotransplantation) has been established.

The use of animals as a source of organs might allow the transplant procedure to be planned, providing obvious medical benefits. In addition, the transplant might be used for the expression of extrinsic genes, as a vehicle for gene transfer. The most suitable source of organs and tissues might intuitively be non-human primates such as chimpanzees and baboons [1,2,27]. However, pigs are now recognized to be the most suitable non-human sources of organs in the future, because of the capability of producing genetically modified pigs (i.e. α-1,3-galactosyltransferase gene knockout pig) [10,21,29] as well as their reproduction-related features, such as early sexual maturity, short gestation time, and generation of large litters [38].

Whether the porcine liver can replace the physiological and anatomical functions of the human liver is a matter of controversy. Porcine livers have been used to provide temporary support for human patients with fulminant
hepatic failure, and devices containing isolated porcine cells are being tested for similar purposes [6]. Although limited information suggests that these approaches improved the well-being of severely ill patients, there is incomplete evidence that they will adequately replace the normal functions of human livers. In part, the limitations may be due to the fact that the mass of the porcine liver and cell systems used to date have been much smaller than the normal human liver [14, 28].

Computed tomography (CT) is frequently used to evaluate graft size preoperatively in both potential recipients and the living donor prior to liver transplantation [15,34]. CT angiography provides information about the liver parenchyma and assesses for the presence of a hepatoma or extrahepatic diseases, as well as determining the patency of the portal vein and the origin and branching patterns of the hepatic arterial system. Accurate knowledge of the hepatic parenchymal and vascular anatomy is crucial to reducing the frequency of complications during and after the transplantation [7,11,36]. The goal of this study was to demonstrate the liver imaging of Yucatan micropigs using the MDCT for assessing liver volume, parenchyma and the vascular anatomy for the selection of suitable donor pigs for liver transplantation.

Materials and Methods

Animals
All experimental procedures were approved by the Ethics Committee of the Chonnam National University. The studies were performed using healthy Yucatan micropigs, all of which were purchased from PWG Genetics (Korea). Prior to their purchase, the pigs were physically examined and confirmed to be healthy. The pigs were housed indoors in individual cages, fed dry pig food freely and provided water. The mean age of the micropigs was approximately 360 days. The mean body weight for the micropigs (male: 2, female: 5) was 34.00 ± 1.74 kg.

Radiological assessment of the liver
The micropigs were deprived of food 24 h prior to the MDCT scan. On the day of the scan, the micropigs were sedated with midazolam (0.1 mg/kg BW) intramuscular injection (i.m.) at neck. After 10 to 15 min, full anesthesia was induced with xylazine (8 mg/kg BW i.m.) and Zoletil 50 (125 mg tiletamin and 125 mg zolazepam; Virbac Animal Health, France) (4 mg/kg BW i.m.), and normal saline was infused through an 18-G venous access line installed in an ear vein. Thereafter, vecuronium bromide (Nocuron 4 mg/vial; Han Hwa Pharma, Korea) (0.1 mg/kg BW) was injected to abolish the autonomic respiration through the line installed in an ear vein. The micropigs were endotracheally intubated and ventilated (250 ml, frequency 10 to 12 per min) during the entire experiment and ventilation was stopped during the MDCT image acquisition. Furthermore, the animal was placed on a heating pad and covered by a blanket and sheets to maintain body temperature. CT examinations were performed using a 16-detector row CT scanner (Sensation 16; Siemens, Germany). Images were acquired from the thorax to the pelvis in a craniocaudal direction with a 0.75 × 16 beam collimation during maintenance of ventrodorsal position. The MDCT scanner was set at a 1.0-mm section thickness, with a gantry rotation time of 500 msec, a table speed of 24 mm/rotation, a detector collimation of 1.5 mm, and a reconstruction interval of 0.8 mm. The tube current was 140 mAs at 120 kVp.

Unenhanced MDCT scanning was performed first and began at the top of the thorax and continued in a craniocaudal direction. After acquisition of unenhanced images, fifty milliliters of contrast medium with a concentration of 320 mg of iodine per milliliter (Visipaque 320; Amersham Health, England) was injected into an ear vein using a power injector (LF CT 9000; Liebel- Flarsheim, USA) at a rate of 2.5 ml/sec. Determination of the scanning delay for the arterial phase imaging was achieved by using an automatic bolus tracking technique (Siemens, Germany). Single-level monitoring low-dose scanning (120 kVp, 20 mAs) was initiated four seconds after contrast material injection. Contrast material enhancement was automatically calculated by placing the region of interest cursor over the vessel of interest (descending thoracic aorta), and the level of the trigger threshold was set at an increase of 40 HU. Two seconds after the trigger threshold had been reached the arterial phase scanning began automatically. The dynamic images consisted of three phases (i.e., arterial, portal venous, and delayed venous).

Image post-processing
Thin-section axial images were transferred to a workstation that had a PC-based three-dimensional (3D) program installed (Rapidia; INFINITT, Korea). Individual volume data were loaded into the 3D program, and the data were reformatted into routine 3D images, which included maximum intensity projection (MIP), multi-planar reconstruction (MPR) and volume-rendered images. The routine MIP images and volume rendered images were reconstructed to cover the thorax to the pelvis in a coronal plane and sagittal plane. Curved MPR was performed by setting the curve axis along each of the arteries in focus. The radiologist performed additional reconstructions, if special focused images were needed after a review of the axial CT scans.

Image analysis
The volume of the liver parenchyma was calculated by serial volumetric assessment from the serial CT scans with semimanual software (Rapidia; INFINITT, Korea). To compare the data between micropigs and human, the total
volume and the right lobe volume of the liver parenchyma were evaluated, because usually the right lobe is transplanted in partial liver transplantation case human-to-human. The diameters of the common hepatic, proper hepatic artery and portal vein were measured on the axial images at the PC-based workstation.

### Statistical analysis
Statistical analysis was carried out with the Statistical Package for the Social Sciences software (SPSS 12.0 for Windows; SPSS, USA). Pearson’s correlation was used to analyze the relationship between body weight and liver volume. A $p$-value $< 0.05$ was considered significant.

### Results
The CT images of the liver parenchyma are illustrated in Fig. 1, and the total liver and right lobe volumes were calculated (Table 1). The liver parenchyma of the micropigs showed homogenous enhancement, similar to humans, and had no space-occupying lesions (Fig. 1).

Anatomically, the proper hepatic arteries originated from the common hepatic arteries and bifurcated to the right and left hepatic arteries as the sole supply of arterial blood to the liver, and there has no variation between micropigs. The mean diameters of the common hepatic artery and proper hepatic artery were 6.24 ± 0.20 mm and 4.68 ± 0.13 mm, respectively. In addition, the mean diameter of the portal vein was 11.27 ± 0.38 mm.

The mean total and right lobe volume of the liver was 698.57 ± 47.81 ml and 420.14 ± 26.70 ml, which were 51.74% and 49.35% of the human total and right lobe liver volume, respectively. For the micropigs, the percentage of liver volume to body weight was approximately 2.05% and there was a significant relationship between body weight and liver volume ($p < 0.05$). The axial CT images of the common hepatic artery, proper hepatic artery and portal vein are shown in Fig. 2. The virtual three-dimensional liver image of the hepatic vascular system reconstructed with serial CT images is shown in Fig. 3, and the diameter of common hepatic artery, proper hepatic artery, and portal vein were estimated (Table 1).

### Discussion
Liver transplantation is currently the only definitive treatment for end stage liver disease. [4, 5] Imaging plays a central role in living-donor transplantation programs by assessing whether potential donors are eligible candidates for liver donation based on anatomical considerations, and whether co-existing pathology is present [25]. Thus, an accurate assessment of the liver anatomy and hepatic vascular variants are essential for successful surgery [25], the determination of the prognosis for micropigs used for xenotransplantation, as well as individual patients.

Rapid technological advances in cross sectional imaging have led to non-invasive techniques, such as CT and magnetic resonance imaging (MRI) that can be used to assess liver volume and vascular anatomy.

### Table 1. Liver parameters in micropigs as measured by multi-detector row computed tomography

| No. | Sex | Weight (Kg) | Volume of liver (ml) | Volume of Rt lobe liver (ml) | Diameter of *CHA (mm) | Diameter of *PHA (mm) | Diameter of *PV (mm) |
|-----|-----|-------------|----------------------|-----------------------------|-----------------------|----------------------|---------------------|
| 1   | M   | 34.5        | 681                  | 472                         | 6.3                   | 4.5                  | 10.2                |
| 2   | M   | 41.5        | 905                  | 501                         | 6.6                   | 4.8                  | 13                  |
| 3   | F   | 38          | 820                  | 351                         | 6.7                   | 5.3                  | 12                  |
| 4   | F   | 30          | 695                  | 336                         | 6.5                   | 4.5                  | 11                  |
| 5   | F   | 28          | 575                  | 418                         | 5.1                   | 4.3                  | 10.8                |
| 6   | F   | 33          | 663                  | 497                         | 6.4                   | 4.5                  | 11.6                |
| 7   | F   | 33          | 551                  | 366                         | 6.1                   | 4.9                  | 10.3                |

Mean ± SD 34.00 ± 1.74 698.57 ± 47.81 420.14 ± 26.70 6.24 ± 0.20 4.68 ± 0.13 11.27 ± 0.38

*CHA; common hepatic artery, PHA; proper hepatic artery, PV; portal vein.
resonance imaging (MRI), replacing conventional angiography for routine evaluation of the hepatic vascular anatomy [8,22,23]. The determination of standard values for the micropig liver, using MDCT, which is also used in human liver evaluations, would be helpful for selecting a suitable porcine donor for an individual patient by determining the condition of the micropig liver, and would also help predict prognosis of the patient.

Imaging evaluation of the liver parenchyma is performed to detect abnormalities such as steatosis, hematomas and hemangiomas [25]. The presence of hepatic steatosis, if in significant quantity, can cause postoperative graft dysfunction in the recipient and liver dysfunction or failure in the donor [3]. Although imaging studies using CT and MRI scanning can detect the presence of hepatic steatosis, the accuracy in quantifying the degree of steatosis continues to be a controversial issue [17,30,31]. In this study, the enhanced CT images showed no evidence of space-occupying lesions such as hemangiomas, hematomas and hepatomas in the liver parenchyma. None of the images acquired were unenhanced CT images. However, the CT images obtained on all micropigs studied showed a relatively homogenous enhancement of the liver. Consistent with previous reports which demonstrate that the normal human liver parenchyma revealed the homogenous enhancement [25, 34], our findings might indicate no significant difference between human and micropig images.

Conventional catheter angiography is the traditional standard reference technique for vascular evaluation; however, it has the drawback of being an invasive procedure [8]. Consequently, the MDCT has replaced conventional angiography for routine evaluations of the hepatic vascular anatomy [8,22,23]. In addition, several studies reported that the analysis of the hepatic vasculature using MRI and CT have a diagnostic accuracy comparable to catheter angiography and excellent intra operative correlation [13,24,34]. In all of the micropigs in this study, the proper hepatic arteries originated from the common hepatic arteries and bifurcated to the right and left hepatic arteries as the sole supply of arterial blood to the liver. The common hepatic arteries measured 5 mm or more in diameter. If the size of these vessels were less than 2∼3 mm in diameter, the patients would be at an increased risk for thrombosis after transplantation [16]. The portal veins were also measured to be 10 mm or more in diameter. Vessel diameter is related with complications such as vessel obstruction or stenosis in liver transplantation. Thus, the measured values indicate that the micropigs hepatic vascular has a sufficient diameter for anastomosis during liver xenotransplantation.

Accurate volume estimation of the liver is essential for the selection of suitable micropigs as a liver donor. In human to human liver transplantation, the graft to recipient body weight ratio should be ≥ 0.8% and preferably ≥ 1% [20]. The graft weight to standard liver volume of the recipient should be about 30∼40% [18,35]. Inadequate graft size can lead to the “small-for-size” syndrome, a clinical entity that encompasses graft dysfunction, liver failure and even death [9], suggesting that the liver graft size which is sufficient to support normal function of body is a critical factor for success of liver transplantation.
In this study, the total liver volume was 698.57 ± 47.81 ml and the right-lobe liver volume was 420.14 ± 26.70 ml in the micropigs. The percentage of whole liver volume to body weight measured by CT scanning was approximately 2.05% in micropigs and 2.04 or 2.11% in human [37,39]. Thus, our data suggested that the difference in liver volume between a human and a micropig is likely due to the difference in body weight. In addition, the right-lobe volume accounts for 60.14% of the total liver volume of the micropig and this relationship was similar in humans [34]. Furthermore, there was a significant relationship between body weight and liver volume ($p < 0.05$), which has also been reported in humans [40]. In a previous report estimating the liver volume in six women (age range, 24-48 years; mean age, 36 years) and eight men (age range, 20-42 years; mean age, 31 years), the mean total volume of the liver was 1.349 ml (ranging from 1.040 to 1,716 ml) [34]. Although we can not determine the possibility that the micropigs liver could be functionally altered the human liver, in this study, our data suggest that the total liver volume of micropigs might be sufficient to support the functions of the human liver in terms of the liver volume needed for liver transplantation, because the total liver volume of the micropigs accounts for 51.74% of the human liver. This imaging-based volumetric assessment technique is relatively accurate in estimating the actual graft volume [12,19,32] and has resulted in a significantly improved prognosis of the patient [18,34]. Furthermore, a previous study showed that the both MDCT an MRI are feasible and robust concepts to evaluate the liver volume and parenchyma in potential living human donors [33]. Although there are many barriers to be overcome for the clinical application of xenotransplantation using the micropig as a potential living donor, the results of this study showed that the hepatic volume and vascular anatomy of the micropigs appeared to be sufficient for adequate replacement of the human liver. In conclusion, MDCT was a reliable imaging method for the evaluation of the liver and its vascular anatomy for xenotransplantation using micropigs.

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