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<Original Article>

Establishment of normal reference of radiological morphology of renal artery in mini-pigs by renal angiography

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Abstract: Mini-pigs have been widely employed in preclinical studies to explore new therapeutic strategies for diseases of the human urinary system; however, the normal reference of the renal artery has not been clearly investigated in the mini-pig model. Therefore, we aimed to establish a normal reference of the radiological morphology of the renal artery in mini-pigs by renal angiography via catheterization of the carotid artery. The renal angiographies obtained from 15 mini-pigs were evaluated to determine the orifice from the aorta, facing direction, size and the number of branches of renal arteries. Cranio-laterally facing renal arteries with 2 distal branches were mainly observed in the renal artery of mini-pigs. Both sides of the renal artery presented symmetrical sizes; however, the right renal artery orifice from the aorta was located more cranially than the left counterpart. The results of this study will contribute to radiological diagnosis of the renal artery as well as preclinical studies of mini-pigs.

Keywords: carotid artery catheterization, mini-pig normal reference, radiological morphology, renal angiography, renal artery intervention

Introduction

The kidney is located in the retroperitoneal cavity and is placed nearby the first to second lumbar vertebrae (L1-L2) level alongside the vertebrae in mammals. Each kidney is connected with the renal artery and vein for blood circulation as well as the ureter to excrete urine into the urinary bladder. Since the renal artery is one of main branch originated from the abdominal aorta, approximately one third of the cardiac output circulates through the renal artery for blood filtration. Therefore, the disorder of the renal artery and the kidney may give rise to systemic symptoms due to its important function with respect to the removal of waste products of metabolism, regulation of electrolytes and blood pressure, and hormone and enzyme production [9, 12].

Because the renal arteries are connected from the aorta to each kidney, the renal artery intervention has been extensively used for the radiological diagnosis of disease in the renal artery and kidney to check the morphological changes via vascular catheterization [4]. The renal artery intervention was commonly achieved by access via the femoral catheterization in most cases. However, the use of thicker vessels were often required due to the limitation of size for application of large intravascular medical device via the femoral artery [4].

Diverse positions and morphologies of the renal artery and kidney were reported depending on different species among the reptile, bird, mammal and human [15]. The location and size of the kidney of mini-pig were clearly addressed by means of excretory urography. In addition, the size of the kidney was well established under ultrasonography [1, 7]. However, the radiological morphology of the renal artery using the renal angiography in mini-pig still remains unclear. Because mini-pig has been widely employed in the preclinical study due to its anatomical, physiological and genetic similarities with the human, normal reference of the radiological morphology of the renal artery in mini-pig should be undoubtedly prepared in advance so as to properly diagnose morbid status and evaluate the morphological changes in the preclinical study. Therefore, the present study was aimed to establish normal reference of the radiological morphology of the renal artery in mini-pig by the renal angiography via the carotid artery catheterization with application of large intravascular medical device, originally developed for human use.

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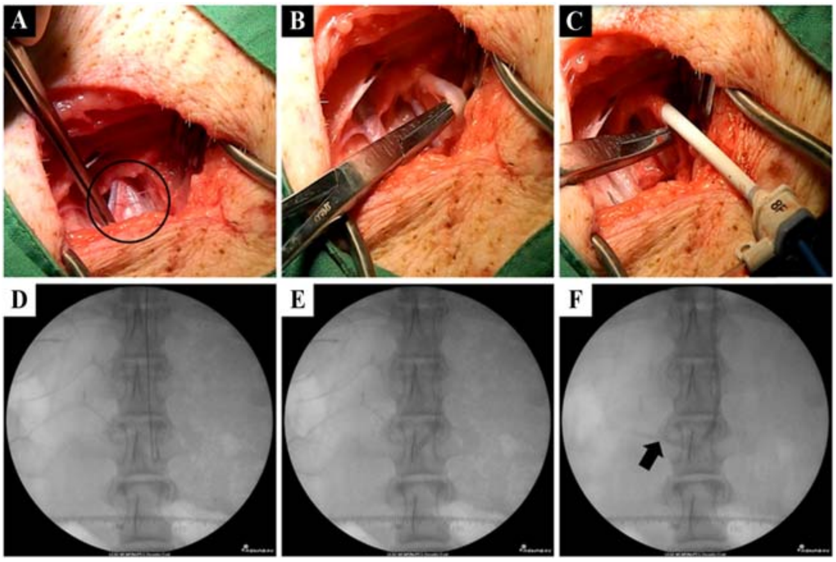

Fig. 1. The carotid catheterization and the renal angiography in mini-pig. (A) Subcutaneous tissue was bluntly dissected till the carotid sheath was observed. Circle indicates the carotid sheath. (B) The carotid artery was gently isolated, and was secured by transverse clamping under the carotid artery. (C) A 7 Fr renal double curve shaped guide catheter was advanced through the 8 Fr introducer sheath. (D) A 0.035 inch guide wire was advanced into the aorta. (E) A 7 Fr renal double curve shaped guide catheter was placed in the aorta. (F) Arrow indicates the seeking the renal artery with injection of small amount of contrast.

Materials and Methods

Ethics and animals

All procedures in regards to the preparation and operation of animals were approved by Institutional Animal Care and Use Committees (IACUC; NP15025). A total number of 15 healthy 18- to 36-month-old female mini-pigs, weighing 33.7 ± 2.5 kg, (PWG Genetics, Singapore) were employed in the present study. Mini-pigs were group-housed and were maintained at 20 to 28ºC on a 12 h light and dark cycle with a daily feeding of standard pig pellet.

Surgical procedure

Each animal was sedated by intramuscular injection of ketamine (10 mg/kg) and xylazine (2.5 mg/kg), anesthetized by 2% isoflurane inhalation in oxygen, and positioned as dorsal recumbent posture. Anesthesia was monitored via a patient monitor (Dräger, Germany) for continuous monitoring of electrocardiography (ECG), heart rate, respiratory rate and oxygen saturation during the surgical procedure. Prior to the surgery, a 16 Fr siliconized Foley catheter for the urinary catheterization (Unomedical, Denmark) was applied to prevent possible overload in the urinary system because the renal angiography accompanies several fluid injections such as heparinized saline, normal saline and contrast. A 5 cm longitudinal cutaneous incision from the sternal manubrium toward cranial direction was made 3 cm away from median plane. The subcutaneous tissue was bluntly dissected till the carotid sheath was observed (Fig. 1A). The carotid artery was gently isolated by trimming the internal jugular vein and vagus nerve from the carotid sheath, was elevated and was secured by transverse clamping under the carotid artery (Fig. 1A).

Exposed carotid artery was cannulated using an 18 G × 45 mm BD Venflon Pro Peripheral IV Catheter (Becton, Dickinson and Company, USA), after which the needle was discarded. The catheter was withdrawn upon the insertion of a 0.025 inch J guide wire (Argon Medical Devices, USA) through the catheter. The 8 Fr introducer sheath and dilator (Medtronic, USA) were advanced over the J wire, and then the dilator with the J guide wire was discarded. A 0.035 inch guide wire (Medtronic) was advanced through the introducer sheath and was placed in the descending aorta under C-arm X-ray guidance (Genoray, Korea) (Fig. 1D). A 7 Fr renal double curve shaped guide catheter (Medtronics) was replaced with the 0.035 inch guide wire (Fig. 1C and 1E). Five hundreds IU/mL of heparin mixed with isotonic saline was used as the catheter lock solution so as to prevent blood clots in the catheter. Once the renal artery ostium was sought by angiography with Ultravist contrast injection (300 mg I/mL; Bayer Healthcare, Germany), the guide catheter was approached into the orifice of the renal artery to capture angiography for further investigation (Fig. 1F). The incision site was closed and all the animals were treated with administration of antibiotics (Baytril; Bayer Healthcare) for 3 days. All animals were closely observed to monitor possible complications for 3 months.

Evaluation of radiological morphology

The renal angiographies were evaluated to investigate the orifice from the aorta, facing direction, size and the number of branch of the renal arteries. To clarify the orifice from the aorta and facing direction of the renal artery, each vertebral column was subdivided as cranial and caudal parts (Fig. 2).

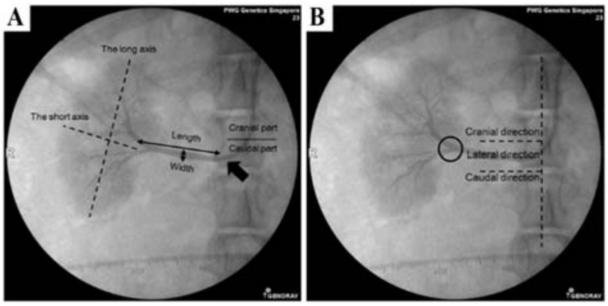

Fig. 2. Evaluation of the radiological morphology of the renal artery and the kidney. (A) The arrow indicates the location of the renal artery orifice. The length and width of the renal artery were measured from the orifice of the renal artery to the renal hilum of the kidney. The dotted lines were regarded as the long and short axis of the kidney. (B) The renal artery in the dotted area is an example of laterally facing renal artery. Circle indicates the presence of branch of the renal artery at the distal region.

The subdivided vertebral column where the renal artery branched from the aorta under the renal angiography was considered as the orifice of the renal artery (Fig. 2A). When the imaginary line was drawn along with the renal artery from the orifice of the renal artery to the renal hilum, it was determined as laterally facing renal artery if the line belonged to an area of subdivided vertebral column; when the end of line was out of the area of subdivided vertebral column toward cranial or caudal direction, it was considered as cranially or caudally facing renal artery, respectively (Fig. 2B). The size of the renal artery was measured for its length and width under observation of the renal angiography (Fig. 2A). The number of the renal branches in the distal region was counted if there is distinguishable branch just before the renal artery went in the kidney (Fig. 2B). In addition, the size of the kidney was easily observed during the renal angiography. Therefore, the length of the long axis across superior and inferior extremity, and the short axis between the renal hilum and lateral margin of both kidneys were measured (Fig. 2A).

Statistical analysis

Student's *t*-test was used to analyze significant differences of each value by PASW software (SPSS, USA). Data are presented as the mean ± SD. The p value less than 0.05 was considered as significant difference.

Results

Postoperative observation

The renal intervention was successfully performed in the total number of 15 animals, and all animals did not show possible postoperative complications such as the artery rupture, seizure and stroke during monitoring periods. The carotid artery was determined to be enough to contain the medical device up to 8 Fr.

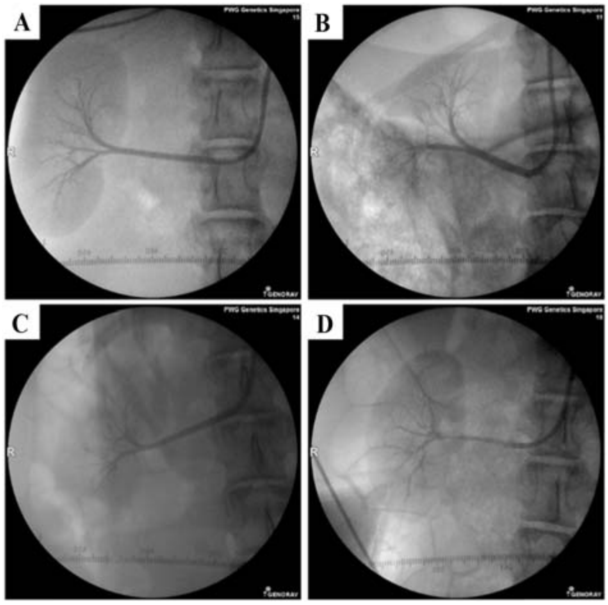

Fig. 3. Representative images of the renal angiography. (A) Laterally facing renal artery with 2 branches was observed. (B) Image of cranially facing renal artery with 2 branches was obtained. (C) Caudally facing renal artery with 2 branches was presented. (D) Laterally facing renal artery without branch was displayed.

Representative images of the renal angiography

Representative images of the renal angiography in mini-pig were displayed in Figure 3. Laterally facing renal artery with 2 branches in the distal region was presented in Figure 3A. In addition, Figure 3B or 3C indicated cranially or caudally facing renal artery with 2 distal branches, respectively. Absence of distal branch of the renal artery could be rarely observed (Fig. 3D).

Normal reference of radiological morphology of the renal artery

The morphological investigation of the renal artery in mini-pig was conducted by evaluating the renal angiography and was summarized in Table 1. The length of long and short axis from both sides of the kidney was not significantly different; the right renal artery showed a tendency of longer length (46.8 ± 7.3 mm) than the left renal artery (41.3 ± 8.5 mm). However, it was not significantly different. But the position of the renal artery orifice from the aorta tended to be asymmetrical; the orifices in the right renal arteries were distributed more cranially across caudal part of the first lumbar vertebrae (caudal L1) to caudal part of the second lumbar vertebrae (caudal L2). The left counterpart placed from cranial part of the second lumbar vertebrae (cranial L2) to cranial part of the third lumbar vertebrae (cranial L3). The renal arteries mainly faced toward cranio-lateral direction in most of cases (14/15 on the both sides) with 2 branches at the distal region (10/15 on the right side; 11/15 on the left side).

Table 1. The incidence frequency of each finding from a total number of 15 animals

	Right side	Left side
The renal artery size		
Length (mm)	46.8 ± 7.3	41.3 ± 8.5
Width (mm)	5.6 ± 1.0	5.8 ± 0.9
The position of the orifice of the renal artery		
Cranial L1	0 / 15	0 / 15
Caudal L1	4 / 15	0 / 15
Cranial L2	7 / 15	5 / 15
Caudal L2	4 / 15	9 / 15
Cranial L3	0 / 15	1 / 15
The renal artery faced toward:		
Cranial direction	4 / 15	7 / 15
Lateral direction	10 / 15	7 / 15
Caudal direction	1/ 15	1 /15
The number of the renal artery		
Single renal artery	15 / 15	15 / 15
Multiple renal artery	0 / 15	0 / 15
The number of the renal artery branch in distal region		
No branch	2 / 15	1 / 15
2 branches	10 / 15	11 / 15
3 branches	3 / 15	3 / 15
The kidney size		
Long axis (cm)	7.2 ± 0.5	7.0 ± 0.6
Short axis (cm)	3.6 ± 0.3	3.5 ± 0.4

The renal artery and the kidney sizes were presented as mean ± SD.

The size of the kidney by renal angiography

The size of the both sides of kidney showed symmetrical manner (Table 1). The long axis of the right or left kidney was determined as 7.2 ± 0.5 cm or 7.0 ± 0.6 cm, respectively. The short axis in the right kidney was 3.6 ± 0.3 cm, and the left counterpart was 3.5 ± 0.4 cm. Overall, there was no significant difference of the long and short axis across each side of the kidney.

Discussion

Because the proper use of laboratory animal for research is necessary to develop new therapeutic strategy and medical device for the human care, porcine has been increasingly highlighted as an animal model in the field of vascular study due to the anatomical and hemodynamic similarities with the human [5]. In particular, porcine urinary system is able to share similarities with that of the human in regards to the structure and anatomical relationships [8]. In addition, their body size provides the opportunity for application of similar surgical procedure with the human, which can be directly transferable to the human.

Since guide wire assisted vascular cannulation was first introduced by Seldinger in 1953, this procedure has extensively employed in the medical science and preclinical study [11]. The external jugular vein is commonly employed to gain intravascular access for repeated dosing or blood collection in the preclinical study of the pharmacology and toxicology. Furthermore, the femoral artery is widely used as an access route in case of blood pressure measurement or medical device approach into main arteries of the heart, liver and kidney through the aorta [3, 4]. However, because the route of catheterization is mainly dependent on the purpose, type of medical device and patient, the use of alternative vessel is sometimes required. The size of the femoral artery of neonate was not enough to contain intra-arterial medical device. Therefore, the umbilical artery catheterization was chosen as an alternative route to approach to the aorta for the treatment of critically ill neonates [6]. In addition, cannulation of the carotid artery has been well applied to access to the aorta when circulatory arrest is necessary, and has shown adequate arterial return without showing any complication at the cannulated site in the total number of 100 human patients [13]. In case of porcine, surgeons cannulated the femoral artery by using a 7 Fr intravascular medical devices in 33 kg pigs [14]. We also tried to cannulate the femoral artery using 8 Fr introducer sheath which is able to contain a 7 Fr medical device at the first time but failed to advance the 8 Fr medical device through the femoral artery in the most cases of mini-pig (data not shown). However, the carotid artery in mini-pig was fully enough to contain an 8 Fr medical device without showing any relevant complications such as the artery rupture, seizure and stroke after surgery.

Varied morphologies of the renal artery in the human have been reported with respect to the incidence of additional renal arteries and presence of exceptional position; the total incidence of multiple renal arteries was reported that one or two additional arteries were observed in a frequency of 23.2% or 4.5% from the human kidney, respectively [10. 15]. Unlikely to the human, single renal artery was more common in porcine with the primary division of the renal vasculature into a cranial and caudal branches (93.4%) [8]. In addition, a comparative gross anatomical analysis was performed across several species and human; approximately 67.4% of the right kidney and 83.7% of the renal artery orifice showed more caudal position than the left counterpart in the human, in contrast, all of the right kidneys and 66.7% of the right renal artery orifice located more cranially than the left side when a total 21 kinds of mammals were comparatively examined [15]. In agreement with those reports, mini-pigs mainly had a single renal artery with distal 2 branches. Most cases of the renal angiography in the present study provided that both sides of the renal artery primarily faced toward cranio-lateral direction. In addition the position of the orifice of the right renal artery from the aorta tended to locate cranially than the left counterpart.

In case of the size of the kidney in mini-pig, the size was known as symmetrical manner in both left and right sides; it was addressed as 8.5 ± 0.6 cm of the long axis and 4.3 ± 0.4 cm of the short axis when excretory urography was applied [1]. In addition, ultrasonography in mini-pig suggested that the length or width of the kidney was 7.0–7.1 cm or 2.6–2.8 cm in 20 week old premature mini-pig, respectively [7]. In agreement with these previous studies, the present study also showed similar size of the kidney as well as no significant difference between both sides of the kidney (Table 1).

The renal artery stenosis causes the restriction of blood supply into the kidney and is manifested of symptoms such as high blood pressure refractory to drug treatment, increased serum creatinine, proteinuria or anuria, changes in body fluid balance and size discrepancy between both kidneys [12]. Angioplasty is known for the recommendable therapy of the renal artery stenosis that narrowed vessel is stretched with a balloon under the radiological guidance to improve blood flow [5]. Furthermore, the renal artery intervention can be applied for diagnosis of the renal vascular abnormalities such as renal artery aneurysms and renal arterio-venous malformations [2]. Likewise, the renal artery intervention and angiography may contribute to diagnostic bio-imaging as well as a therapeutic application. Furthermore, there have been several trials in regards to the renal artery advance in porcine model to overcome the human disease, for instance, new therapeutic strategy by direct delivery of stem cells in atherosclerotic renal artery stenosis porcine model, and application of newly developed medical device into porcine vessel for the renal denervation to treat a hypertensive patient [3, 9].

We explored the radiological morphology of the renal artery in mini-pig by means of the renal angiography, and can expect that the present study may contribute to secure normal reference data to diagnose morbid and abnormal renal artery in the radiological observation as well as safety of the renal intervention procedure via the carotid artery.

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