International Scholars Journals

International Journal of Urology and Nephrology ISSN 2091-1254 Vol. 7 (2), pp. 001-004, February, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

A novel rat model of $^1/_4$ partial nephrectomy and its application in ischemia-reperfusion research in residual kidney

Qianglin Duan[1, 2], Guangchun Wang[2] and Junhua Zheng[2*]

[1]Department of Scientific Research, Tongji HospitalTongji University, No. 389 Xincun Road, Shanghai 200065, China.
[2]Department of Urology, Tenth People's Hospital, Tongji University, No. 301 Yanchang Road, Shanghai 200072, China.

Accepted 11 June, 2010

This study established a new rat model of $^1/_4$ partial nephrectomy for research on ischemia-reperfusion injury in residual kidney. Thirty healthy Sprague-Dawley rats were intraperitoneally anesthetized by pentobarbital sodium. A lumbar oblique incision was made, and then the right renal pedicle was isolated and clamped by a smooth microclip. The kidney size was measured and the upper pole of the right kidney (about 0.5 × 1.0 cm) was resected by a scorching knife and then SURGICEL* FIBRILLAR Absorbable Hemostats (oxidized regenerated cellulose) was used for rapid haemostasis. Mean kidney size was 2.0 × 1.1 cm, and mean kidney tissue removed actually was 0.6 × 1.0 cm. Mean operation time was 3.2 ± 0.5 min (2.6 - 5.2 min), and mean haemostasis time after resection was 1.2 min (0.8 - 1.5 min). Three minutes after partial nephrectomy, the smooth microclip was removed for reperfusion, without rebleeding in all cases. No rats died during the operation. A new $^1/_4$ partial nephrectomy rat model was successfully established in our study, which can be used for research on ischemia-reperfusion injury in residual kidney. Rapid haemostasis could be achieved by SURGICEL* FIBRILLAR Absorbable Hemostats, which is suitable for application in surgical experiments in rats.

Key words: Partial nephrectomy, ischemia-reperfusion injury, haemostasis, rat model.

INTRODUCTION

So far, there are still tens of related research articles on renal ischemia-reperfusion injury published in some famous journals such as <J Urol> (Yossepowitch et al., 2006), <Urology> (Orvieto et al., 2005), <Int J Urol> (Humphreys et al., 2009) and <Transplantation> (Harper et al., 2008), etc, indicating that renal ischemia-reperfusion injury is still a hot topic in urological studies. However, regardless of human investigations or animal experiments, most of the studies on renal ischemia-reperfusion injury were carried out in intact kidneys, and only few results were from residual kidneys following small-part partial nephrectomy (Huri et al., 2009; Secin, 2008). A more suitable rat model of $^1/_4$ partial nephrectomy for research on ischemia-reperfusion injury in residual kidney has not been reported.

*Corresponding author. E-mail: zhengjh0471@163.com. Tel: +8621-66307478.

MATERIALS AND METHODS

Animals

Thirty healthy Sprague-Dawley rats, aged 8 - 10 weeks, weighing 250 – 300 g, were purchased from the Experimental Animal Center of the Chinese Academy of Sciences and fed in the Animal Center of Tongji Hospital, Tongji University (Shanghai, China). All rats were food- and drink-fasted for 6 h before surgery. All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of Tongji University, and conformed to the National Institute of Health guidelines on the ethical use of animals.

Partial nephrectomy and rapid haemostasis

After being intraperitoneally anesthetized by 2 ml of pentobarbital sodium solution (0.001 kg/L), animals were placed in the left latericumbent position. After skin unhairing and sterilizion by 75% (v/v) alcohol, a lumbar oblique incision under the right costal margin was made to expose the right kidney, which was then pulled ventralward. After separating the perirenal fat, the right renal

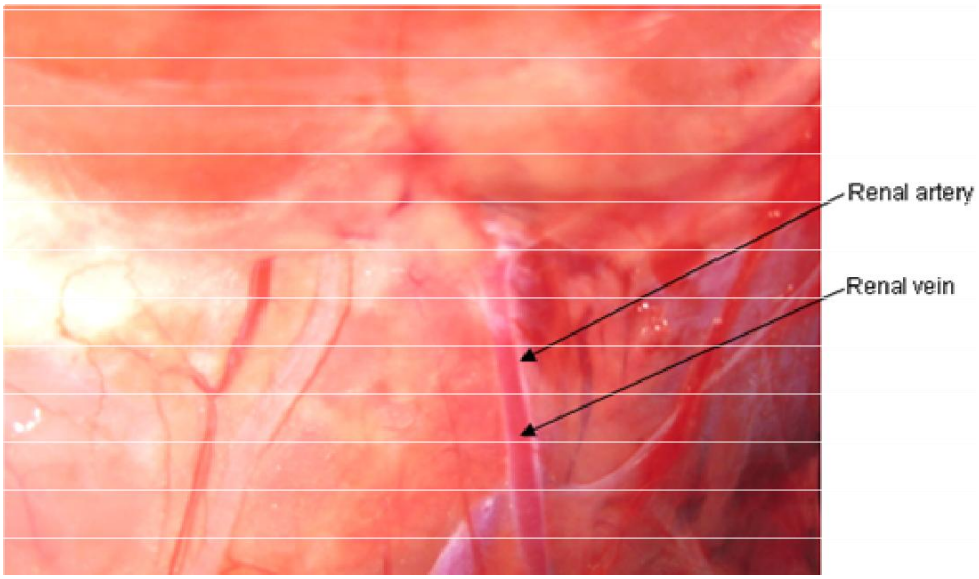

Figure 1. Anatomic structure of the right renal hilum.

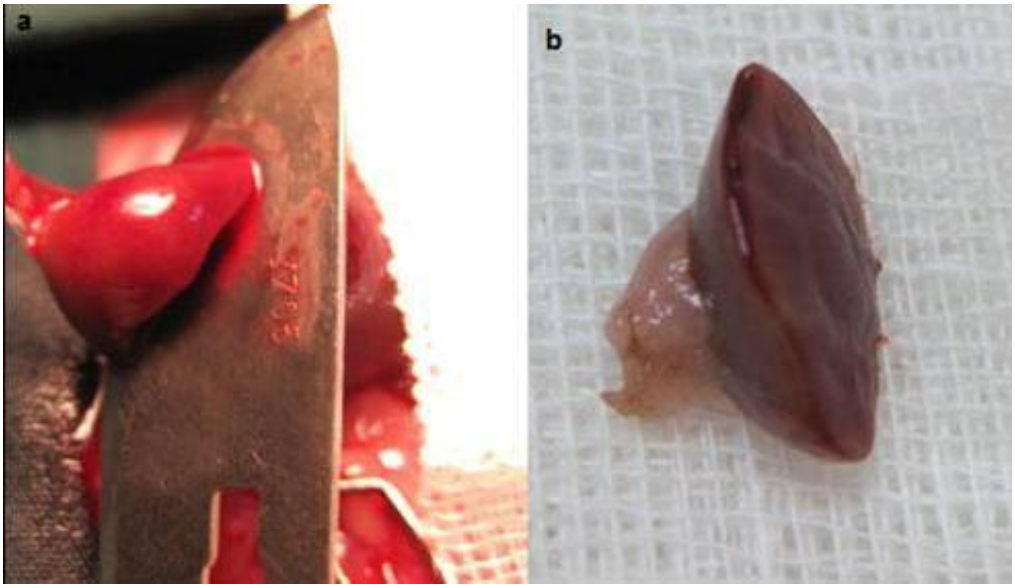

Figure 2. $^{1}/_{4}$ partial nephrectomy in the upper pole of the right kidney. (a) $^{1}/_{4}$ partial nephrectomy in the upper pole of the right kidney with a scorching knife, (b) the kidney tissue removed.

pedicle was exposed.

The renal artery was accompanying with renal vein and the artery was located in the thoracic side of renal vein in all cases (Figure 1). The right renal pedicle was clamped by a smooth microclip, and the kidney size was measured, including the maximal transverse diameter and maximal longitudinal diameter. Then the upper pole of the right kidney, about 0.5 ×1.0 cm, was removed by a scorching knife (Figure 2), and then a piece of SURGICEL* FIBRILLAR Absorbable Hemostats (oxidized regenerated cellulose) was used for rapid haemostasis (Figures 3a and b). Three minutes after partial nephrectomy, the smooth microclip was removed for reperfusion, and no wound surface rebleeding was observed in any case (Figure 3c). No rats died during the operation.

RESULTS

Mean kidney size was 2.0 × 1.1 cm, and mean kidney tissue removed actually was 0.6 × 1.0 cm. The removed kidney tissue was approximately 1/4 of the primary kidney size. Mean operation time was 3.2 ± 0.5 min (2.6 5.2 min). The knife was preheated and small vessels could be coagulated while resection, and then a piece of SURGICEL* FIBRILLAR Absorbable Hemostats (oxidized regenerated cellulose) was used to cover the wound for rapid haemostasis. Mean haemostasis time after resection

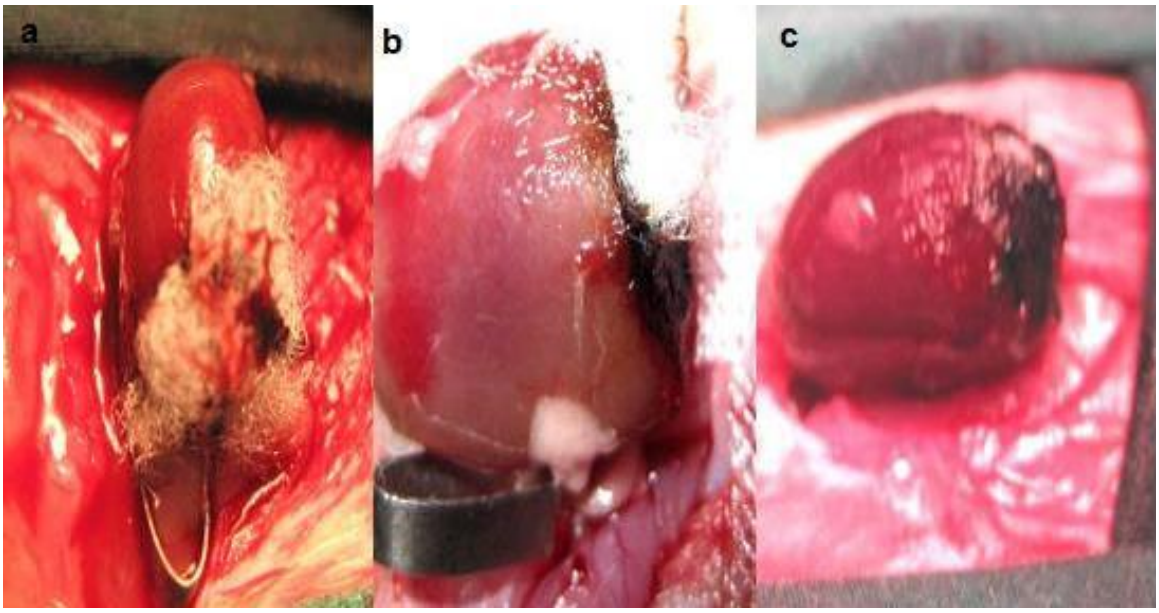

Figure 3. Rapid haemostasis with the SURGICEL* FIBRILLAR absorbable hemostats.

resection was 1.2 min (0.8 - 1.5 min). Three minutes after partial nephrectomy, the smooth microclip was removed for reperfusion, without rebleeding in all cases. No hemorrhoea occurred during the modeling procedure. No rats died during the operation. 24 h after the modeling procedure, all the modeling rats survived, with a survival rate of 100%.

Our modeling rats have been used for investigating the effects of different warm ischemia times (WITs) including 20, 30, 40, 50, 60 min on ICAM-1, bcl-2 and bax mRNA and protein in residual kidney. Eighty healthy rats were randomly divided into renal pedicle clamping group and renal artery clamping group. After clamping for 20, 30, 40, 50 or 60 min (n = 8), the microclip was relaxed for renal reperfusion. Another 8 healthy rats served as control (WIT0). After ischemia-reperfusion for 24 h, resi-dual renal sample was obtained by right nephrectomy, and then the protein and mRNA expressions of ICAM-1 in rat residual renal tissue were determined by immunohistochemistry and RT-PCR. Compared with the weakly positive expression of ICAM-1 protein in WIT0, WIT20 and WIT30 groups, strongly positive expression of ICAM-1 protein was present in WIT40, WIT50 and WIT60 groups, with significant difference ($p < 0.05$). The mRNA expression level of ICAM-1 increased progressively from WIT0 group to WIT60 group, however, significant differences could be found between WIT30 and WIT40 groups ($p < 0.05$), WIT30 and WIT50 groups ($p < 0.01$) as well as WIT30 and WIT60 groups ($p < 0.01$), respectively, but there was no significant difference between WIT20 and WIT30 groups, indicating that renal injury was mild if WIT was less than 30 min. With the extension of WIT, renal cell injury showed an increasing and aggravating trend, however, WIT of less than 30 min has little effect on renal tissue, suggesting that it is safe for partial nephrectomy.

DISCUSSION

Over the years, many studies on renal ischemia-reperfusion injury have been available (Troncoso et al., 2005; Kakkar et al., 2004; Laven et al., 2004; Huri et al., 2009; Phull et al., 2008), and it has always been the research focus in the urological field. However, these studies have three major features.

First, most of the data about renal ischemia-reperfusion injury were from solitary kidney model, because the animals underwent contralateral nephrectomy before grouping (Harper et al., 2008; Baldwin et al., 2004). The solitary kidney model was indeed more suitable for studying the effects of ischemia time on kidney function or related molecular biological changes. However, for patients who underwent partial nephrectomy clinically, most have a healthy or normal contralateral kidney, so, studies on renal ischemia-reperfusion injury should be based on the animal model with a normal contralateral kidney, which is more consistent with the actual situation of clinical status. The rat model we designed has a normal contralateral kidney, so, it will be valuable in future experiments.

Second, the modeling kidney is always intact, not partially resected in most animal experiments (Troncoso et al., 2005; Kakkar et al., 2004; Laven et al., 2004), so it is necessary to investigate the effects of ischemia-reperfusion injury on the residual kidney tissue following partial nephrectomy, because partial nephrectomy instead of radical nephrectomy has been the treatment of

choice for patients with early-stage renal cell carcinoma. Partial nephrectomy involves only cancer tissues and normal renal parenchyma is preserved. It is an excellent surgical procedure especially for patients with solitary kidney or contralateral renal insufficiency. Previously, open partial nephrectomy is a standard of care for small exospheric renal masses. With the development of laparoscopic surgery, laparoscopic partial nephrectomy has become the frequently used technique in the treatment of patients with renal cell carcinoma. In the treatment of complex cancers, the kidney will experience long-time ischemic injury. Especially in the laparoscopic partial nephrectomy in which the procedure is compli-cated, shorter ischemia time may affect the surgical operations and prolonged ischemia time may result in the aggravation of warm ischemia injury. Thus, it is imperative to explore the effects of warm ischemia time on the residual kidney and thus optimize it

Third, the $^{5}/_{6}$ partial nephrectomy model was the most commonly used animal model for research on partial nephrectomy (Fleck et al., 2006), but is only limited for studies on kidney failure. However, partial nephrectomy is mainly applied for treatment of superficial small renal tumors of less than 4 cm, during which, only about less than 30% of kidney tissues will be removed in most cases so, a small-part partial nephrectomy animal model should be established for studies on ischemia-reperfusion injury. Only few reports about small-part partial nephrectomy rat or murine model are available on studies on effects of ischemia-reperfusion injury following partial nephrectomy (Huri et al., 2009; Phull et al., 2008).

By using a simple operative procedure and haemostasis method, a new rat model of $^{1}/_{4}$ partial nephrectomy which was not previously reported was successfully established in our present study. It will be more suitable and valuable for future research on effects of ischemia-reperfusion injury in residual kidney following small-part partial nephrectomy. It can be used to determine the effect of different warm or cold ischemia time on kidney function and molecular biological changes in the residual kidney. On the basis of animal models previously described, we investigated the effects of warm ischemia time on the expression of ICAM-1, an inflammatory factor in rats that underwent small-part partial nephrectomy. Our results indicated that WIT of less than 30 min was safe and tolerant for residual renal tissues. However, optimized cold ischemia periods and the effects of laparoscopy on the safe ischemia periods are required to be further investigated in future studies.

ACKNOWLEDGEMENT

This study was supported by the Novel Technology Project of Multiple General Hospitals in Shanghai (SHDC12010104).

REFERENCES

Baldwin DD, Maynes LJ, Berger KA, Desai PJ, Zuppan CW, Zimmerman GJ, Winkielman AM, Sterling TH, Tsai CK, Ruckle HC (2004). Laparoscopic warm renal ischemia in the solitary porcine kidney model. Urology, 64: 592-597.

Fleck C, Appenroth D, Jonas P, Koch M, Kundt G, Nizze H, Stein G (2006). Suitability of 5/6 nephrectomy (5/6NX) for the induction of interstitial renal fibrosis in rats--influence of sex, strain, and surgical procedure. Exp. Toxicol. Pathol., 57: 195-205.

Harper SJ, Hosgood SA, Waller HL, Yang B, Kay MD, Goncalves I, Nicholson ML (2008). The effect of warm ischemic time on renal function and injury in the isolated hemoperfused kidney. Transplantation, 86: 445-451.

Humphreys MR, Castle EP, Lohse CM, Sebo TJ, Leslie KO, Andrews PE (2009). Renal ischemia time in laparoscopic surgery: an experimental study in a porcine model. Int. J. Urol., 16: 105-109.

Huri E, Akgül T, Ayyildiz A, Ustün H, Germiyanoğlu C (2009). Hemostatic Role of a Folkloric Medicinal Plant Extract in a Rat Partial Nephrectomy Model: Controlled Experimental Trial. J. Urol., 181: 2349-2354.6.

Troncoso P, Ortiz AM, Domínguez J, Kahan BD (2005). Use of FTY 720 and ICAM-1 antisense oligonucleotides for attenuating chronic renal damage secondary to ischemia-reperfusion injury. Transplant. Proc. 37: 4284-4288.

Kakkar AK, Lefer DJ (2004). Leukocyte and endothelial adhesion molecule studies in knockout mice. Curr. Opin. Pharmacol., 4: 154-158.

Laven BA, Orvieto MA, Chuang MS, Ritch CR, Murray P, Harland RC, Inman SR, Brendler CB, Shalhav AL (2004). Renal tolerance to prolonged warm ischemia time in a laparoscopic versus open surgery porcine model, J. Urol., 172: 2471-2474

Orvieto MA, Tolhurst SR, Chuang MS, Lyon MB, Ritch CR, Rapp DE, Shalhav AL (2005). Defining maximal renal tolerance to warm ischemia in porcine laparoscopic and open surgery model. Urology, 66: 1111-1115.

Phull H, Lien YH, Salkini MW, Escobar C, Lai LW, Ramakumar S (2008). Delivery of intercellular adhesion molecule-1 antisense oligonucleotides using a topical hydrogel tissue sealant in a murine partial nephrectomy/ischemia model. Urology, 72: 690-695.

Secin FP (2008). Importance and Limits of Ischemia in Renal Partial Surgery: Experimental and Clinical Research. Adv. Urol., 102461.

Yossepowitch O, Eggener SE, Serio A, Huang WC, Snyder ME, Vickers AJ, Russo P (2006). Temporary renal ischemia during nephron sparing surgery is associated with short-term but not long-term impairment in renal function. J. Urol., 176: 1339-1343