Duplex scan and histologic assessment of acute renal injury in a kidney-kidney crosstalk swine experimental model

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ABSTRACT

Objective: The objective of this study was to identify the effect of two left renal vasculature occlusion strategies on the duplex ultrasound-assessed rheology and histology of the contralateral kidney.

Methods: Pigs were randomly assigned to one of two groups: left renal artery-only clamping (A group, n = 8) or left renal artery and vein clamping (AV group, n = 9). Bilateral renal parenchymal biopsy specimens were taken every 10 minutes for 90 minutes. Duplex ultrasound resistive index (RI) and pulsatility index (PI) were measured. Mixed models with normal distribution and first-order autoregressive correlation structure and generalized estimating equation models were used. Results are presented as adjusted means with standard errors, estimated proportions with standard errors, and line plots with 95% confidence intervals.

Results: RI and PI increased in the nonischemic kidney. In A group animals, RI values increased significantly ($P < .01$) after 30 minutes of ischemia and PI increased significantly ($P < .04$) from 30 to 60 minutes of ischemia. The number of histologic abnormalities was higher in A group than in AV group biopsy specimens. The percentage of lesions increased significantly after 10 minutes in A group nonischemic kidneys ($P < .02$) and between 50 and 80 minutes in AV group nonischemic kidneys ($P < .01$).

Conclusions: Nonischemic kidneys were acutely affected by contralateral ischemia. Their function was more adversely affected by unilateral renal artery occlusion with preserved renal vein patency (A group). (J Vasc Surg 2017;■:1-8.)

Clinical Relevance: Our finding that the nonischemic kidney experienced acute effects stemming from contralateral ischemia may help surgeons prevent acute kidney injury during vascular and urologic procedures. Moreover, simultaneous artery and vein clamping appeared to affect the occurrence of renal crosstalk, which was reflected in worsening perfusion, elevated resistive index and pulsatility index values, and a tendency for thrombi to form in control kidneys. In addition, renal clamping protocol affected the occurrence of histologic lesions.

Acute kidney injury (AKI) secondary to surgery may progress to definitive disease, depending on the severity and duration of the injury.[1-3] The "safe" duration of clamping is controversial, and few studies have examined the acute behavior of the contralateral kidney during renal ischemia using duplex ultrasound scan parameters. Experimental studies have shown that AKI may be associated with the onset of lesions in distant organs, such as the liver, lungs, and brain.[4] The renal damage caused by hypoxia triggers an inflammatory process that may provoke a systemic reaction, resulting in pathophysiologic sequelae in distant organs in a process called kidney-organ crosstalk.[4,5] However, the occurrence of kidney-kidney crosstalk and the potential damage of this inflammatory cascade to the contralateral kidney remain poorly understood.[6-8]

Importantly, there is no consensus in the literature on the relationship between increased AKI risk and clamping protocol. In fact, the advantages of using either renal artery-only clamping, maintaining venous flow and some renal perfusion, or artery and vein clamping, with total flow obstruction and reduced risk of bleeding, are not clearly defined.[9,10]

The use of vascular Doppler ultrasound for the analysis of renal perfusion and graft control is well established.[11,12] Measurements of the resistive index (RI), pulsatility index (PI), and Doppler spectral pattern provide valuable information about renal parenchymal vascularity.[11,13] Thus, several experimental animal models have been developed to detect changes in renal perfusion using Doppler ultrasound.[14] In this study, we serially measured RI and PI to determine the effects of ischemia on the contralateral kidney and compared the relative impact of renal artery alone vs renal artery and vein clamping on renal crosstalk.

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METHODS

The study was conducted between January and December 2016 at the Center for Experimentation and Training in Surgery of the Albert Einstein Jewish Hospital, São Paulo, Brazil, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The study was approved by the Albert Einstein Jewish Hospital Animal Care and Use Committee. A completed Animal Research: Reporting In Vivo Experiments guidelines checklist is included in the Appendix (online only).

Seventeen 3- to 4-month-old female Large White pigs weighing between 29 and 35 kg were randomly assigned to either a renal artery and vein (AV group, n = 9) or artery-only (A group, n = 8) clamping protocol. Ischemia-induced AKI was experimentally reproduced through either left artery and vein or artery-only clamping, with the right kidney serving as a nonischemic control. Renal biopsies and ultrasound assessment were performed bilaterally at time 0 and every 10 minutes up to 90 minutes of ischemia. Duplex ultrasound scan analysis and biopsies were also performed during kidney reperfusion, at 120 and 150 minutes into the surgical procedure.

Preoperative anesthetic procedures. All pigs were preanesthetized intramuscularly with ketamine (10.0 mg/kg) and midazolam (0.25 mg/kg). The marginal ear vein was catheterized with a 22-gauge BD Insyte catheter (BD Infusion Therapy Systems Inc, Sandy, Utah) for venous access. Anesthesia was induced with thiopental (7 mg/kg), and fluid replacement was performed with a maintenance crystalloid solution at 10 mL/kg/h. Crystalloid solution at 1 to 2 mL/kg/h was administered in bolus form whenever necessary to maintain a mean blood pressure of at least 70 mm Hg. Size 7.0 Portex endotracheal tubes (Smiths Medical, Ashford, UK) were used, and inhalational anesthesia was achieved with 2% isoflurane with the ventilator set at a tidal volume of 10 mL/kg. Anesthesia was maintained with fentanyl (2.5 μg/kg). Continuous invasive arterial pressure monitoring was accomplished by arterial catheterization of the right femoral artery. The mean blood pressure in each group is presented in Table I. Blood pressure was maintained at a minimum of 70 mm Hg during the procedure. Animals with nonresponsive hypotension were excluded. Systemic heparin (150 units/kg) was administered before hilar clamping. Even though the use of heparin in urologic surgery is controversial, systemic heparinization was employed because of the susceptibility of pigs to thrombi.[15,16] The use of vasoactive agents was not allowed, and hypotension was managed with volume replacement. After surgery, all pigs were humanely euthanized by a lethal dose of thiopental and 15 to 30 mg/kg of 19.1% potassium chloride.

ARTICLE HIGHLIGHTS

- **Type of Research:** Experimental swine model of unilateral renal ischemia
- **Take Home Message:** In vivo physiologic and histologic data support that significant unilateral kidney ischemia causes changes in the contralateral nonischemic kidney.
- **Recommendation:** The nonischemic kidney may suffer damage by unclear mechanisms when one kidney is critically ischemic.

Table I. Adjusted mean values and standard errors for the mean blood pressure in each group

	Clamping protocol	
Time, minutes	A group (n = 8)	AV group (n = 9)
Basal	101.4 (2.3)[a]	94.9 (2.2)
10	94.7 (2.0)[b]	93.6 (1.9)
20	87.9 (2.5)[a,b]	94.7 (2.4)
30	80.9 (2.4)[a,b]	89.7 (2.3)
40	78.1 (2.6)	80.4 (2.4)[b]
50	78.1 (2.6)[b]	79.9 (2.4)[b]
60	73.1 (3.0)[b]	70.6 (2.8)[b]
70	76.6 (3.4)[a,b]	66.4 (3.2)[b]
80	76.1 (3.6)[b]	72.4 (3.4)[b]
90	72.9 (4.2)[b]	74.2 (4.0)[b]

A group, Artery-only clamping; *AV group*, artery and vein clamping. The data represent the adjusted mean values and standard errors calculated using generalized estimating equation models.
[a]Significant difference between groups.
[b]Significant difference from baseline.

Operative technique. The kidneys were accessed through a midline laparotomy from xiphoid to pubis. The kidneys were biopsied bilaterally at time 0. Next, left renal hilar clamping was achieved using bulldog forceps, and serial parenchymal biopsies were performed in both kidneys. Biopsy samples were then gently collected using a No. 11 ophthalmic scalpel blade, with no significant parenchymal injury or major blood loss. Reperfusion was initiated after 90 minutes.

Ultrasound data. Ultrasound examination was performed using a Philips HD7 ultrasound system (Koninklijke Philips N.V., Amsterdam, The Netherlands) and multifrequency linear transducer (7-11 MHz) in two-dimensional, color, pulsed, and power Doppler modes. Image registration was performed at time 0 to rule out any previous alterations. Power Doppler ultrasound was used whenever necessary to confirm the absence of flow.

Ultrasound analysis. The RI and PI were calculated automatically by the ultrasound software (High Q