Different from Renal Artery Only Clamping, Artery and Vein Clamping Causes a Significant Reduction in Number of Rat Glomeruli During Warm Ischemia

Hélio J. S. Bagetti-Filho, D.V.M., Ph.D.,[1] Francisco J. B. Sampaio, M.D., Ph.D.,[1]
Ruy G. Marques, M.D., Ph.D.,[2] and Marco A. Pereira-Sampaio, D.V.M., Ph.D.[1,3]

Abstract

Purpose: To evaluate glomerular injury in the rat model during renal warm ischemia (WI), comparing artery and vein (AV) clamping with artery only (AO) clamping.

Materials and Methods: Twenty-four adult male rats underwent 60 minutes of renal WI in the left kidney. The animals were divided into three groups: AV clamping, AO clamping, and Sham surgery. After 30 days, the animals were euthanized, and both kidneys were processed for paraffin embedding and stained with hematoxylin and eosin. Glomerular volume density (Vv[glom]), mean glomerular volume (MGV), and number of glomeruli per mm^3 (Nv[glom]) were evaluated in the renal cortex.

Results: The Vv[glom] was reduced in the left kidney (ischemic) when compared with the right kidney in both AV and AO groups by 11.1% and 35.4%, respectively; however, the difference was significant only in the AV group. The Nv[glom] was reduced in the left kidney when compared with the right kidney in both AV and AO groups by 11.6% and 31.4%, respectively; nevertheless, the difference was significant only in the AV group. The MGV of left and right kidneys was the same in both Sham and AO groups and was diminished by 6.7% in the AV group—not significant.

Conclusion: AV clamping causes a significant decrease in the number of glomeruli in the rat model, while AO clamping reduces the glomerular number, but not significantly. To minimize renal injury, AO clamping may be preferred over AV clamping when WI is necessary in patients with previously compromised renal function.

Introduction

RENAL WARM ISCHEMIA (WI) is commonly used in laparoscopic partial nephrectomy (LPN), but parenchymal damage still is the main concern during the perioperative period[1] and is the most frequent cause of chronic[2] and acute[3–5] renal failure. Renal ischemia is also responsible for an increased risk of rejection in renal transplantation.[5,6]

Ischemia-reperfusion causes endothelial damage, oxidative stress, and increased inflammatory response, which leads to several renal injuries.[1] The release of free radicals, as superoxide and hydroxyl, is an important mechanism of cell injury in tissues that are subjected to ischemia-reperfusion.[7] Mechanisms of protection of ischemic renal injury are of utmost importance to better outcomes in nephron-sparing surgery.[2]

Renal WI can be performed using artery only (AO) clamping[3,8,9] or artery and venous (AV) clamping together.[4,7,10,11] Although Plaine and Hinman[12] found no difference in renal function between AO and AV occlusion in rabbits, other studies in the porcine model demonstrated that the AV occlusion is more harmful for renal function, suggesting that the open renal vein enables retrograde perfusion and avoids venous congestion of the kidney.[13–15] Renal vein only occlusion in rats resulted in a more severe functional injury than AO occlusion.[1] These studies, however, used only functional tests to assess the effects of warm ischemia during AO and AV clamping.

The morphological evaluation was performed by subjective scores, usually considering brush border loss, tubular cell necrosis, cellular vacuolation, and tubular epithelial loss.[16] Until now, the renal parenchyma was not quantified to evaluate the real and long term damage caused by AO compared with AV clamping. Recently, it was demonstrated in the porcine model that the glomerular density decreases in the remaining renal parenchyma after LPN.[17]

Mean time of AO occlusion of 43 minutes during LPN seems to have no negative effect on renal function, challenging traditional concepts of maximum renal ischemia of 30

[1]Urogenital Research Unit and [2]Department of Surgery, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.
[3]Department of Morphology, Fluminense Federal University, Niteroi, RJ, Brazil.

minutes. Despite these encouraging results, these studies also evaluated the renal function using nuclear renography and serum creatinine analysis.[9] Renal WI of 25 to 45 minutes has been used in several experiments to assess the renal damage in rats.[1,3,4,7,8] Park and colleagues,[1] however, reported that recovery after 30 minutes of renal ischemia was complete; 90 minutes of ischemia caused high mortality, while 60 minutes of renal ischemia produced severe renal tubular necrosis, acceptable mortality, and residual morphologic damage.

The aim of this study was to evaluate, by using unbiased stereologic methods, the effects of two methodologies of vascular clamping (AO vs AV) in a rat model after 60 minutes of renal warm WI.

Materials and Methods

Twenty four male Wistar rats, 4 months old, were used. They were divided into three groups of 8: (1) AO clamping; (2) AV both clamped; and (3) Sham (S) operated.

The rats were anesthetized via intramuscular ketamine 100 mg/kg and xylazine 20 mg/kg, prepped for surgery, and a ventral midline incision was used to expose the abdominal contents, which were displaced to the right side. The left renal AV were isolated. One of the three procedures (AO, AV, S) was performed: AO—the renal artery was occluded with a bulldog clamp for 60 minutes; AV—the renal AV en $bloc$ were clamped for 60 minutes; or Sham—vessels were isolated, but no clamp was applied. The abdominal contents were replaced, and the incision was covered with moistened gauze during the ischemic period. At the end of the ischemic period (60 min), the clamps were removed, and reperfusion was observed. Finally, the abdominal wall was closed.

All experiments were performed according to the national law for scientific use of animals, and this project was formally approved by the local Ethics Committee for animal experimentation.

All animals were euthanized 30 days after surgery by intravenous injection of a high dose of pentobarbital. Both left and right kidneys were harvested and fixed in 10% buffered formaldehyde. Renal volume was estimated by the Scherle method,[18] and the proportional area of the cortex and medulla was calculated by the Cavalieri method[19] using Image-J software. Random samples from all 48 kidneys were processed for paraffin embedding, sectioned at 5-μm thickness, and stained with hematoxylin and eosin. From each kidney, 25 histologic fields obtained from five different sections of the renal cortex were examined. Glomerular volume density (Vv[glom]), which indicates the volume occupied by the glomeruli in the cortex, was estimated by the point-counting method.[19]

The mean glomerular volume (MGV) was estimated by using the "point-sampled intercepts method."[19] analyzing five microscopic fields per section and five sections per kidney. Randomly, crossed glomerular length was performed with a 32-mm long logarithmic ruler composed of a series of 15 classes. Each individual intercept was cubed, and the mean of all values was multiplied by $\pi/3$ in every case to obtain the MGV.[20]

The number of glomeruli per cubic millimeter of renal cortex (Nv[glom]) was calculated by dividing the Vv(glom) by the MGV.

Animals were weighed 1 day before surgery, on postoperative days 3 and 30, and correlated with the renal volume.

The analysis of variance with Bonferroni[21] post-test was used for stereologica data mean comparisons. Animal weight and renal volume correlation was assessed by linear correlation. For all comparisons, $P < 0.05$ was considered significant. The analyses were performed using GraphPad Prism software.

Results

The AO Vv(glom) was 14.65% (range 11.19–18.58); the AV Vv(glom) was 12.02% (range 3.81–19.80); and the S Vv(glom) was 16.03% (range 8.61–21.86) in left kidneys. In right kidneys, the AO Vv(glom) was 16.48% (range 12.13–22.20); the AV Vv[glom] was 18.60% (range 15.16–22.49); and the S Vv(glom) was 16.09% (range 8.59–25.41). The Vv(glom) was reduced in left kidneys (ischemic) when compared with right kidneys in both AO and AV groups by 11.1% and 35.4%, respectively; however, the difference was significant only in AV ($P = 0.0074$). Furthermore, there was no difference between left and right kidneys from the Sham group (Fig. 1).

The AO Nv(glom) was 13.89 glomeruli/mm^3 of renal cortex (range 11.13–18.65); the AV Nv(glom) was 12.34 glomeruli/mm^3 of renal cortex (range 4.06–20.50); and the S Nv(glom) was 14.74 glomeruli/mm^3 of renal cortex (range 7.15–19.91) in left kidneys. In right kidneys, the AO Nv(glom) was 15.72 glomeruli/mm^3 of renal cortex (range 11.75–19.58); the AV Nv(glom) was 17.98 glomeruli/mm^3 of renal cortex (range 14.98–22.92); and the S Nv(glom) was 15.10 glomeruli/mm^3 of renal cortex (range 9.09–23.55). The Nv(glom) was reduced in left kidneys (ischemic) when compared with right kidneys in both AO and AV groups by 11.6% and 31.4%, respectively; nevertheless, the difference was significant only in AV ($P = 0.0167$) (Fig. 2). Moreover, there was no difference between left and right kidneys from the Sham group (Fig. 1).

The AO MGV was 1.07 $10^3 \mu$m^3 (range 0.80–1.34); the AV MGV was 0.97 $10^3 \mu$m^3 (range 0.80–1.17); and the S MGV was 1.10 $10^3 \mu$m^3 (range 0.85–1.30) in left kidneys. In right kidneys, the AO MGV was 1.06 $10^3 \mu$m^3 (range 0.77–1.29); the AV MGV was 1.04 $10^3 \mu$m^3 (range 0.85–1.27); and the S MGV was 1.07 $10^3 \mu$m^3 (range 0.94–1.15). The MGV of left kidneys was almost the same of the right kidneys in both Sham and AO groups, and diminished by 6.7% in the AV group—not significant (Table 1).

Discussion

Despite the advanced development of LPN techniques, which can mimic almost completely open surgery,[22] renal cooling techniques to minimize renal damage by WI are not easily performed during laparoscopic surgery.[23] Recently, ice-slurry has been tested in a porcine model for renal hypothermia during LPN, but it has still not gained clinical acceptance.[24] Bleeding control during renal surgery can be accomplished in several ways, but vascular occlusion is still very important for reducing bleeding in the surgical field, enabling accurate tumor resection and precise collecting system closure.[25] Vascular occlusion results in a transient period of WI that may affect renal function, however.[2]

Because the main purpose of conservative renal surgery is to preserve the largest number of nephrons and because the glomeruli cannot be produced after birth, an objective evaluation of the number of glomeruli after WI is of great importance to compare AO and AV occlusion. Such is the
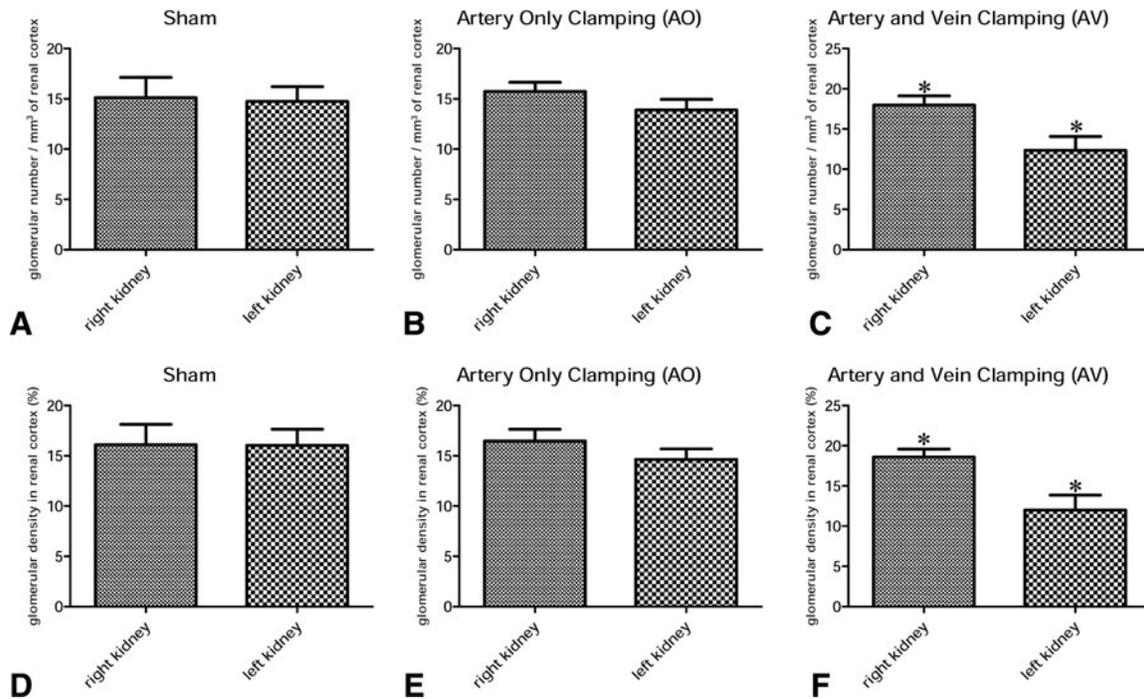
FIG. 1. Number of glomeruli per mm^3 of renal cortex in both right and left kidneys from group Sham **(A),** group Artery Only clamping **(B),** and group Artery and Vein clamping **(C).** Glomerular density in renal cortex in both right and left kidneys from group Sham **(D),** group Artery Only clamping **(E),** and group Artery and Vein clamping **(F).** The results are expressed as mean and standard error. *$P < 0.05$.

unbiased stereologic method used in this study, which can accurately estimate the number of glomeruli in the kidney.[19]

In the current study, Vv(glom) and Nv(glom) in the renal cortex were significantly reduced in left (ischemic) kidneys when compared with right (nonoccluded) kidneys of AV groups by 35.4% 11.1 and 31.4% 11.6, respectively. Even though there was reduction of Vv(glom) and Nv(glom) in AO left kidneys (11.1% and 11.6%, respectively), the differences was not significant ($P = 0.2603$ and 0.2147, respectively). Furthermore, there was no difference between left and right kidneys from the Sham group (Fig. 1). To our knowledge, the present morphologic study demonstrated for the first time that the AV clamping caused more structural damage in the renal parenchyma than the AO clamping in rats. Recently, it was demonstrated that the AV occlusion during LPN in a

porcine model significantly reduced the number of glomeruli in the renal parenchyma.[17] This difference is more important when estimated for the remaining parenchyma of a partial nephrectomy, where a portion of the kidney has been removed.

Apoptotic bodies can be found in the distal renal tubules after 5 minutes of AO occlusion within 48 hours of reperfusion in rats. After 30 minutes of occlusion, the apoptosis spreads for all renal tubules, and after 45 minutes the apoptosis spreads throughout the kidney with increasing of tubular necrosis.[8] Ysebaert and colleagues[26] demonstrated in rats undergoing 60 minutes of AV clamping that the first signs of regeneration appeared at the second day of reperfusion, and at the 10th day of reperfusion, half of the proximal tubules were completely regenerated. These findings support the
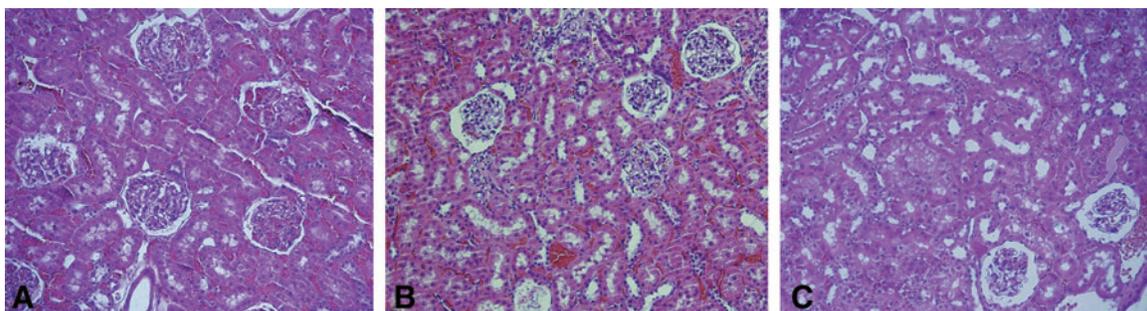

FIG. 2. Photomicrographs of renal cortex of left kidneys from group Sham **(A),** group Artery Only clamping **(B),** and group Artery and Vein clamping **(C).** Note a reduced number of glomeruli in group Artery and Vein clamping when compared with group Sham and group Artery Only clamping. Hematoxylin and eosin, X200.

Table 1. Stereologic Data of Kidneys from Animals Undergoing Artery Only Clamping, Artery and Vein Clamping, and Sham Surgery

	Artery only clamping			Artery and vein clamping			Sham		
	Right	Left	P value	Right	Left	P value	Right	Left	P value
MGV	1.06 (±0.16)	1.07 (±0.19)	0.8533	1.04 (±0.12)	0.97 (±0.13)	0.2381	1.07 (±0.09)	1.1 (±0.16)	0.6368
Vv(glom)	16.48 (±3.28)	14.65 (±2.95)	0.2603	18.6 (±2.82)	12.02 (±5.23)	0.0074	16.09 (±5.77)	16.03 (±4.55)	0.9806
Nv(glom)	15.72 (±2.62)	13.89 (±3.01)	0.2147	17.98 (±3.2)	12.34 (±4.92)	0.0167	15.1 (±5.72)	14.75 (±4.12)	0.8883

Data expressed as mean ± standard deviation.
MGV = mean glomerular volume; Vv(glom) = glomerular volume density; Nv(glom) = number of glomeruli per cubic millimeter of renal cortex.

estimating of glomerular number as a more reliable parameter for assessing the structural renal injury after ischemia/reperfusion, because the tubular injury can regenerate, while glomeruli will not regenerate. Therefore, the decreasing of the glomerular number suggests a permanent damage of the renal parenchyma.[27,28]

AO and AV clamping have been studied in humans with regard to postoperative renal function,[29] and the morphologic changes are also compared between AO and AV occlusion in animal models.[13,15,30] The AO clamping seemed to be less hazardous, preserving the renal morphology. The mechanism of damage prevention in the AO occlusion seems to be related with the venous backflow, decreasing free radicals and capillary thrombosis, and increasing availability of substrate for metabolism and renal oxygenation.[13] Formiga and colleagues,[30] comparing AO, AV, and intermittent occlusion, demonstrated that the intermittent clamping has the advantage of washing out catabolites from anaerobic metabolism and increasing the oxygen saturation in the renal tissue. Tracy and coworkers[13] have shown that the tissue oxygenation is higher in AO occlusion, after 30 to 35 minutes of WI. Therefore, the oxygenation of the renal tissue seems to be the most important difference between the AO and AV occlusion, leading to a significant prevention of renal damage in the AO occlusion.

One of the limitations of this study is the use of a rat model, because of the anatomic differences from human kidneys, such as renal volume, unipapillary kidney, and rudimentary caliceal system. The use of an animal model, however, was imperative to obtain the entire kidney for performing the technique of stereologic analysis. Another limitation of the study is in regard to surgery, because we perform an open surgery instead of a laparoscopic approach, so the impact of the pneumoperitoneum was not assessed. Functional evaluation, as a creatinine clearance, was not used, because we kept the contralateral kidney to use as a control for the kidney that underwent ischemia.

The key to a successful nephron-sparing operation is the preservation of the remaining parenchyma in good functional status. Therefore, the use of all available measures is important for this. Likewise, each patient must be evaluated to choose the most effective treatment.[10] The AO occlusion for 60 minutes did not reduce the number of glomeruli in the rat model. Gong and colleagues[29] demonstrated that the AO clamping did not lead to higher difficulty during LPN; this method of ischemia, then, should be considered when other measures are unavailable, as well as in situations that make the renal cooling difficult, either by perfusion of saline or use of ice-slurry.[24] The AO clamping must also be considered, especially in patients with previous impaired renal function.

Ultimately, however, more studies should be performed regarding the benefit of AO clamping during LPN.

Conclusion

The AO clamping for 60 minutes does not cause significant glomerular damage, while the AV clamping for 60 minutes causes a significant decrease in the number of glomeruli in the rat model. Thus, the AO clamping could be recommended when WI is necessary or in patients with previous compromised renal function, to minimize renal injury.

Acknowledgments

Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Marco A. Pereira-Sampaio, D.V.M., Ph.D.
Rua Comendador Queiroz
37/902, Icaraí
Niterói, RJ
Brazil

E-mail: sampaio@vm.uff.br

Abbreviations Used

AO = artery only
AV = artery and vein
MGV = mean glomerular volume
Nv[glom] = number of glomeruli per mm3
LPN = laparoscopic partial nephrectomy
Vv[glom] = glomerular volume density
WI = warm ischemia