Glomerular loss after arteriovenous and arterial clamping for renal warm ischemia in a swine model[1]

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ABSTRACT

PURPOSE: To evaluate the glomerular loss after arteriovenous or arterial warm ischemia in a swine model.

METHODS: Twenty four pigs were divided into Group Sham (submitted to all surgical steps except the renal ischemia), Group AV (submitted to 30 minutes of warm ischemia by arteriovenous clamping of left kidney vessels), and Group A (submitted to 30 minutes of ischemia by arterial clamping). Right kidneys were used as controls. Weigh, volume, cortical volume, glomerular volumetric density (Vv[Glom]), volume-weighted glomerular volume (VWGV), and the total number of glomeruli were measured for each organ.

RESULTS: Group AV showed a 24.5% reduction in its left kidney Vv[Glom] and a 25.4% reduction in the VWGV, when compared to the right kidney. Reductions were also observed when compared to kidneys of sham group. There was a reduction of 19.2% in the total number of glomeruli in AV kidneys. No difference was observed in any parameters analyzed on the left kidneys from group A.

CONCLUSIONS: Renal warm ischemia of 30 minutes by arterial clamping did not caused significant glomerular damage, but arteriovenous clamping caused significant glomerular loss in a swine model. Clamping only the renal artery should be considered to minimize renal injury after partial nephrectomies.

Key words: Nephrectomy. Warm Ischemia. Kidney Glomerulus. Models, Animal. Swine.

Introduction

The diagnosis of small renal masses has been rising over the past two decades[1], and partial nephrectomy is being increasingly performed to treat this type of tumor[2]. The goal of partial nephrectomy is to remove the tumor while preserving the healthy kidney parenchyma, sparing the maximum possible number of nephrons[3]. For achieving a bloodless field during kidney dissection, commonly the organ is submitted to transient warm ischemia.

Warm ischemia during partial nephrectomy is identified as a factor that negatively influences the postoperative renal function and is the most frequent cause of chronic and acute renal failure[4,5]. Despite some techniques for avoiding the use of warm ischemia during partial nephrectomy[6], the hemostatic control by clamping the renal vessels is still used by several groups[7].

Classically, the renal pedicle was clamped en bloc avoiding the removal of the perivascular adipose tissue. This would save about 30 minutes of intraoperative time for dissecting the vessels, and cushion the renal vessels from the vascular clamp[8]. However, some studies have shown that renal function is better preserved when only the renal artery is clamped for a partial nephrectomy[9-11]. Recently, a study in rats submitted to 60 minutes of kidney warm ischemia demonstrated that arterial only clamping did not impair the renal parenchyma, different from arteriovenous clamping that decreased the glomerular number[12]. Although these studies have shown some advantages on clamping only the renal artery, the impact of this technique for preserving glomeruli in swine is not known.

The aim of this study was to evaluate glomerular losses after arterial only or arterioveneous warm ischemia in a swine model, by using unbiased stereologic methods.

Methods

All experiments were performed in accordance with the Brazilian law for scientific use of animals, and this project was formally approved by the local ethics committee for animal experimentation (CEUA039-2013).

Twenty four male pigs weighing 20kg (mean) were used in this study. The animals were divided into three groups with 8 animals in each: Group Sham was submitted to all surgical steps except the renal ischemia; Group AV was submitted to renal warm ischemia by clamping the renal artery and vein; and Group A was submitted to renal ischemia by only renal artery occlusion.

Under general anesthesia, the animals were placed in right lateral decubitus and a laparoscopic approach with three trocars was used to access the left kidney, as previously described[13]. After identifying and dissecting the renal vessels, a laparoscopic vascular clamp was positioned according to the experimental group. Thus, for group AV the clamp should occlude both artery and vein, for group A only renal artery was occluded, and for group Sham the clamp was positioned but not closed. Animals of groups AV and A were submitted to 30 minutes of left kidney warm ischemia, while animals of group Sham were maintained under anesthesia for the same period. Hereafter, the clamp was removed and reperfusion was observed in the ischemic kidneys. The right kidneys were not manipulated during the experiment and were used as controls. No method for achieving cold ischemia was used.

All animals were submitted to euthanasia 21 days after surgery by anesthetic overdose (pentobarbital). Both left and right kidneys were collected and fixed in 4% buffered formaldehyde. Kidneys were weighed and their volumes were estimated by the Scherle method[14]. The cortical-medullar ratio was estimated by using the Cavalieri principle[15] and the absolute cortical volume (CV) was calculated by multiplying the cortical-medullary ratio by the renal volume.

Fragments of renal cortex were collected from the 48 kidneys and were processed for stereological analysis. The specimens were routinely processed for paraffin embedding, sectioned at 5µm thickness, and stained by hematoxylin & eosin. From each kidney, 25 randomly histological fields of the cortex were analyzed.These fields were photographed with a digital camera (DP70, Olympus, Tokyo, Japan) coupled with a microscope (BX51, Olympus) under the same conditions (magnification x200; resolution 2.040x1.536 pixels) and stored in a TIFF file.

Glomerular volume density (Vv[glom]), which indicates the proportional volume occupied by the glomeruli in the cortex, was estimated by the point-counting technique with a M42 test-system[3,14]. The volume-weighted glomerular volume (VWGV), which indicates the mean volume of each glomerulus, was estimated with the point-sampled intercepts method analyzing 50 glomeruli per animal[3,12]. Analyses of Vv[glom] and VWGV were done using the ImageJ software (version 1.46r, NIH, Bethesda, Maryland, EUA)

The estimation of the total number of glomeruli per kidney was calculated by multiplying the CV by the Vv [glom] and dividing the result by the VWGV[14,15].

Serum creatinine and urea levels were measured in samples obtained before surgery, 10 daysafter surgery, and immediately before euthanasia.

For each morphological parameter, left kidneys were compared to right organs of each group using the Student's *t*-test. Also the left kidneys of the three studied groups were compared using one way ANOVA with Bonferroni's post test. Mean creatinine and urea serum levels were also compared using one way ANOVA. For all comparisons $p<0.05$ was considered significant. Data was expressed as mean ± standard deviation. Analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, USA).

Results

For all groups, the left kidneys' weight and volume were statistically similar to that of right kidneys. Also, no difference was observed when the left kidney of groups Sham, AV and A were compared. Additionally, no difference was present regarding the absolute cortical volume of the studied kidneys.

The Vv[glom] was diminished by 24.5% in kidneys submitted to en bloc clamping, in comparison to the right kidneys of this group. The left kidneys of groups Sham and A were not different from their right controls. When comparing the left kidneys of the three groups, we found that the Vv[glom] of group AV was also 24.5% lower than the Sham group. No difference was found between the left kidneys of Sham and A group (Figure 1).

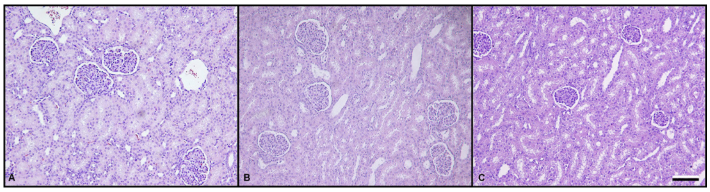

FIGURE 1 - Photomicrographs of cortical fields from kidneys submitted to Sham surgery (**A**) and warm ischemia by arterial (**B**) or arteriovenous clamping (**C**). Hematoxylin and eosin, x200, scale bar represent 100µm.

The VWGV showed similar results. The left kidneys of group AV were 25.4% smaller than its right controls. For the groups Sham and A, left and right organs were similar. When comparing the VWGV among the three groups, it was noted that group AV glomeruli had 24.9% lower volume than those of group Sham. Again, no difference was found between the left kidneys of Sham and A group for this parameter.

When analyzing the number of glomeruli per kidney it was observed that the left kidneys of group AV were the only affected organs, with a reduction of 19.2% in comparison to its contralateral control. Arterial camping did not affect the number of glomeruli as no difference was found when comparing the results of the left kidneys of group A to its right control. All morphological data are listed in Table 1.

There was no significant difference in creatinine and urea serum levels at the different times of withdrawal in the studied groups (Table 2).

TABLE 1 - Stereological data of right and left kidneys of pigs submitted to sham surgery or to left renal ischemia 30 minutes by clamping the renal artery and vein (AV) or the renal artery (A).

	Sham			AV			A		
	Right	Left	*p* value	Right	Left	*p* value	Right	Left	*p* value
Kidney weight (g)	56.9 ± 3.4	56.7 ± 6.9	0.93	56.4 ± 6.9	54.4 ± 7.6	0.60	57.1 ± 3.5	56.9 ± 5.0	0.93
Kidney volume (ml)	55.9 ± 4.0	55.8 ± 7.6	0.98	55.7 ± 5.5	52.2 ± 7.1	0.28	54.9 ± 3.1	54.3 ± 4.9	0.79
Cortical volume (ml)	40.0 ± 3.1	39.5 ± 7.0	0.85	40.1 ± 5.1	36.6 ± 5.5	0.60	39.5 ± 2.1	38.5 ± 4.3	0.93
Vv[glom] (%)	4.4 ± 0.1	4.4 ± 0.2	0.88	4.4 ± 0.1	3.3 ± 0.2*	<0.0001	4.4 ± 0.1	4.4 ± 0.2	0.89
VWGV (10^5 μm^3)	13.9 ± 0.5	13.8 ± 0.8	0.88	13.9 ± 0.5	10.4 ± 1.2*	<0.0001	13.8 ± 0.9	13.6 ± 0.6	0.65
Glomeruli (millions)	1.26 ± 0.1	1.26 ± 0.2	0.98	1.30 ± 0.1	1.05 ± 0.2	0.02	1.26 ± 0.1	1.25 ± 0.2	0.89

*Different from left kidney of Sham group; Data expressed as mean ± S.D.

TABLE 2 - Serum creatinine and urea levels drawn before surgery and on postoperative days 10 and 21 of pigs submitted to sham surgery or to left renal ischemia 30 minutes by clamping the renal artery and vein (AV) or the renal artery (A).

		Preoperative	10 days Postoperative	21 days Postoperative	*p* value
Sham	Creatinine	1.15 ± 0.13	0.95 ± 0.17	1.15 ± 0.29	0.38
	Urea	38.0 ± 1.4	39.2 ± 6.3	37.0 ± 3.0	0.64
AV	Creatinine	1.12 ± 0.31	1.12 ± 0.15	1.09 ± 0.27	0.94
	Urea	38.1 ± 6.1	40.8 ± 5.5	37.4 ± 2.7	0.38
A	Creatinine	1.21 ± 0.19	1.03 ± 0.17	1.14 ± 0.25	0.31
	Urea	39.7 ± 3.7	38.6 ± 7.8	37.7 ± 4.0	0.78

Data expressed as mean ± S.D.

Discussion

In 2007, Spaliviero and Gill suggested that clamping the renal vessels en bloc for laparoscopic partial nephrectomy would have advantages over dissecting the renal pedicle and clamping the renal artery alone[8]. Since then, great progress has been made in studying methods for achieving renal ischemia for nephron sparing surgery. Even so, many groups use different techniques for tumor removal with or without ischemia[7], and clamping the renal artery only or artery and veins remains a controversial point. While some studies found a better preservation of renal function when only the renal artery is clamped[9-12], this is the first study to address the number of glomeruliafter en bloc versus arterial clamping for achieving renal warm ischemia in the pig model.

The mechanisms that explain the advantages of clamping the artery only are still unknown. However, during arterial only ischemia the kidney blood may flow through the renal vein as it is not occluded, decreasing venous congestion. Ischemia-reperfusion process induces oxidative stress and increased inflammatory response, releasing oxygen reactive species in the bloodstream[16]. These molecules should be reduced by antioxidants or may cause cell harm and death[17]. We may suppose that during an en bloc renal ischemia the oxygen reactive species generated become restrained inside the kidney causing harm to the organ with cell death and possibly leading to glomerular losses observed in the present study. On the other hand, when only the artery is clamped, these molecules can flow to bloodstream through the unobstructed renal veins, being reduced by antioxidants.

Orvieto *et al.*[10] observed that clamping only the renal artery during an open partial nephrectomy in a swine model causes less impact on renal function than clamping the renal artery and veins. However, the benefit of clamping only the renal artery was not observed when the laparoscopic approach was used for partial nephrectomy. The authors postulated that the pneumoperitoneum may partially occlude the venous backflow, eliminating the benefits of the isolated arterial clamping[10]. Our study showed different results since (despite the abdominal insuflation) the kidneys submitted to ischemia by clamping only the renal artery had no significant decrease in glomerular number as compared to those submitted to en bloc clamping. Furthermore, it was previously demonstrated in a rodent study, that pneumoperitoneum used under adequate pressures does not cause harm to kidney morphology[15]. Even so, in the aforementioned study[10] the authors used a prolonged warm ischemia time (120 minutes) what could explain the different results observed in ourexperiment.

Berczi *et al.*[18] studying renal function in patients with solitary kidney undergoing partial nephrectomy with renal warm ischemia demonstrated that most patients had chronic renal failure. This may suggest that studies on renal function after partial nephrectomy with warm ischemia in patients with two kidneys may have been affected by patients' renal function being compensated by the contralateral kidney, which suffered no damage. Our study assessed the number of glomeruli, in both the operated kidney and the contralateral kidney, thus avoiding the bias that can occur when renal function is evaluated in patients with contralateral kidney. Furthermore, our results clearly demonstrate that the loss of glomeruli, thereby also the diminished amount of functional nephrons, reduced renal function. This may not be detectable in functional tests, but it is a risk factor in long-term renal function after renal warm ischemia.

Studying the renal ischemia by analyzing the number of glomeruli seems to be a valuable method, as the objective of this surgery is to treat small renal tumors while avoiding the loss of nephrons. Many times, the number of nephrons does not directly correlate with short-term renal function. We have observed that renal function commonly remains unaltered even after a great loss of nephrons[3,14]. However, as these lost nephrons cannot be restored, the individual is more likely to develop renal insufficiency/failure at the long term. This may explain the decrease in renal function observed after some years in patients submitted to renal ischemia for partial nephrectomy[4,19,20].

Future studies investigating the effects of clamping the renal artery or artery and vein are warranted. For instance, it is not known if the better results of the artery-only clamping are seen when cold ischemia is used. Furthermore, if the oxygen reactive species and the oxidative stress are involved in the glomerular loss after ischemia by arterial or en bloc clamping, studies investigating the use of antioxidants would be interesting.

We should point out that this is an animal study and its results should not be directly transposed to humans. Although the swine is the most adequate model for comparison to human kidney anatomy and physiology[13,21], this is still an experimental setting and different from the clinical setting. Further, these animals were healthy individuals, without renal tumor or any other medical condition which, again, does not represents the patients submitted to partial nephrectomy.

Conclusions

In our swine model, the renal warm ischemia of 30 minutes by arterial clamping did not caused significant glomerular damage or nephron loss. By the other hand, when arteriovenous clamping was used, 30 minutes of warm ischemia caused a decrease in the number of glomeruli. Thus, whenever possible, clamping only the renal artery for warm ischemia should be favored over en bloc clamping to minimize renal injury after partial nephrectomies.

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