Kidney Healing After Laparoscopic Partial Nephrectomy Without Collecting System Closure in Pigs

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OBJECTIVES To access the kidney healing after laparoscopic partial nephrectomy without closuring of the collecting system in pigs.

METHODS Fourteen pigs underwent left partial laparoscopic nephrectomy, with removal of 25% of the kidney length at caudal pole (n = 7) or at cranial pole (n = 7). Briefly, the surgical technique involved a transperitoneal laparoscopic access, *en bloc* vascular clamping of renal vessels, tissue excision with cold scissor and monopolar energy hemostasis of only the parenchyma, leaving the collecting system opened, with no insertion of a double-J catheter. The animals were clinically evaluated during 14 days, and afterward were killed. Serum levels of urea and creatinine were assessed prior and at different moments after surgery. Macroscopic necropsy analysis, a retrograde *ex vivo* pyelogram and a histologic study of the operated renal poles were performed.

RESULTS The animals did not show any postoperative clinical alterations. Serum levels of urea and creatinine showed a slight raising at the second postoperative day with gradual decreasing to preoperative levels. At necropsy, the abdominal cavity was normal, with normal quantity and aspect of peritoneal liquid. No signs of urine leakage were found. The operated renal pole was always involved by a perirrenal fibrosis with adherences to adjacent organs.

CONCLUSIONS The pig kidney collecting system healed well without any kind of suture or internal drainage. Therefore, we concluded that the pig kidney is not an adequate model for research on which the collecting system healing is an important aspect to be considered. UROLOGY 77: 508.e5–508.e9, 2011. © 2011 Elsevier Inc.

The pig kidney has been considered the best model for renal surgery because of its anatomical resemblance to the human kidney.[1,2] As such, with the advent and wide use of laparoscopic partial nephrectomy for treating localized renal tumors, this animal model is being largely used for research and training on these novel surgical techniques.[3-5]

The collecting system closure with intracorporeal suture during laparoscopic partial nephrectomy is considered a technically demanding procedure that increases the surgical time and requires longer warm ischemia.[6] Therefore, research has been done for simplifying and accelerating this step.[7-11]

It was previously reported that the pig kidney does not develop urinoma after partial nephrectomy in the polar extremity without collecting system closure[12]; nevertheless, little is known regarding the details of renal healing under this condition. In fact, pigs are still being used as a model for research on collecting system repair.[7] Therefore, the aim of this study was to access kidney healing in pigs after laparoscopic partial nephrectomy without closure of the collecting system.

MATERIAL AND METHODS

Fourteen male domestic pigs, weighting a mean average of 30 kg, were subjected to left partial laparoscopic nephrectomy, with removal of 25% of the kidney total length at the caudal pole (n = 7) or at the cranial pole (n = 7), exposing the caudal or the cranial major calices, respectively.[13]

This project was approved by the local ethical committee in accordance with Brazilian laws for scientific use of animals.

Under general anesthesia and aseptic technique, the surgical procedure involved a transperitoneal laparoscopic access with 4 trocars as an adaptation from the usual technique used in humans.[14] The left kidney was dissected for obtaining its total exposure. The kidney length was measured with a polypropylene tube. Afterward the tube was removed from the abdominal cavity and 25% of it was reintroduced in the abdomen for

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determining the exact segment of cranial or caudal pole to be resected. Renal vessels were *en bloc* clamped, avoiding excessive dissection, and the kidney was incised with cold scissors. The collecting system entering was determined when the surgeon felt a more dense structure to incise. After complete removal of the renal pole region, the opening of the collecting system was confirmed by direct visualization. Monopolar energy was applied for hemostasis only in the parenchyma, avoiding coagulation near the collecting system. Once again the collecting system opening was verified and no internal collecting system drainage catheter was inserted. The excised fragment was removed through an extension of a port size incision. Animals received regular analgesics for 24 hours after surgery. Food and water were given *ad libitum* after recovery of normal ambulation, usually 12-24 hours after the procedure.

The animals were clinically evaluated during 14 days after surgery, and afterward they were killed by anesthetic overdose. Serum levels of urea and creatinine were assessed before surgery and at postoperative days 2, 6, 10, and 14, to assess the renal function and any possible peritoneal absorption resulting from intracavitary urinary leakage. These data were statistically compared by one-way ANOVA considering $P < .5$ to indicate statistical significance.

At the 13th postoperative day, the animals were transfered to individual stalls. Methylene blue was diluted in the drink water for staining the urine to demonstrate any urinary leakage, which would stain the tissue around the kidney.

During necropsy, the peritoneal fluid was collected for urea and creatinine analysis. The abdominal cavity and retroperitonium were evaluated for any evidence of urinary leakage around the operated kidney. Special attention was paid for identification of urinomas, urinary fistulae, peritonitis, and methylene blue extravasation.

The operated kidney was removed, the ureter was catheterized, and an *ex vivo* retrograde pyelogram was performed, to evaluate any leakage of contrast medium. The organ was fixed in 10% formaldehyde, and the operated pole was cleaved for obtaining a fragment of renal tissue together with adhered adjacent tissues. The fragment was processed for paraffin embedding, sectioned at 5-μm thickness and stained with hematoxylin and eosin, Masson's trichrome and Sirius red, for histologic analyses.

RESULTS

The surgical technique used was effective for obtaining an open collecting system model, as it was possible to observe the lumen of the cranial or the caudal major calice in all animals after kidney section.

All animals recovered well after surgery, showing normal function (ambulation, food and water intake, and urination and defecation) within the first 24 hours.

Serum levels of urea and creatinine showed a slight increase in the second postoperative day (not statistically significant), with a gradual decrease to preoperative levels until the end of the experimental period. Even so, these levels remained far below the upper limit of reference levels for pigs (Figure 1).

At necropsy, the abdominal cavity was normal, with normal quantity and aspect of peritoneal liquid. Also, the peritoneal fluid levels of urea and creatinine were similar to the normal serum levels (urea 27.9 ± 2.1 mg/dL; creatinine 0.98 ± 0.08 mg/dL).

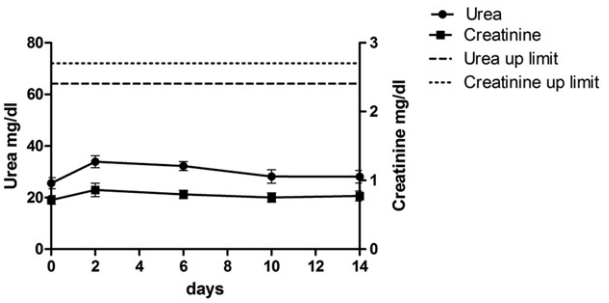

Figure 1. Serum levels of urea and creatinine during the postoperative period.

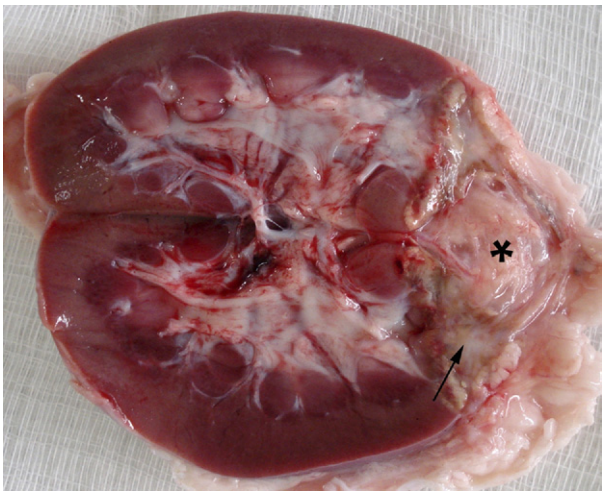

Figure 2. Longitudinal section of a left pig kidney submitted to a caudal pole partial nephrectomy. One may note fibrosis in the operated renal pole sealing the collecting system that was sectioned (arrow). Also shown is the presence of fat firmly adherent to the fibrotic tissue (asterisk).

In all animals, the operated pole was completely covered by fibrous tissue (Figure 2), which hindered the identification of the collecting system. We also observed several adherences of the operated pole to adjacent organs (spleen, colon, and pancreas). The urinary bladder was filled with blue-colored urine; nevertheless, no blue-stained tissue was observed around the kidney. No urinomas, fistulae, or any other signs of urine leakage were found in any animal, also no abnormalities were found in the nonoperated (right) kidney.

The retrograde pyelograms depicted the collecting system anatomy without evidence of any contrast medium extravasation. In some kidneys, subjected to pyelography with injection under high pressure, the contrast medium penetrated the renal parenchyma (collecting ducts) and still did not present any leakage in the operated pole (Figure 3). Interestingly, we have observed some cases of high-pressure pyelograms, on which we observed rupture of collecting ducts and extravasation in the nonoperated pole, whereas the operated pole remained without leakage.

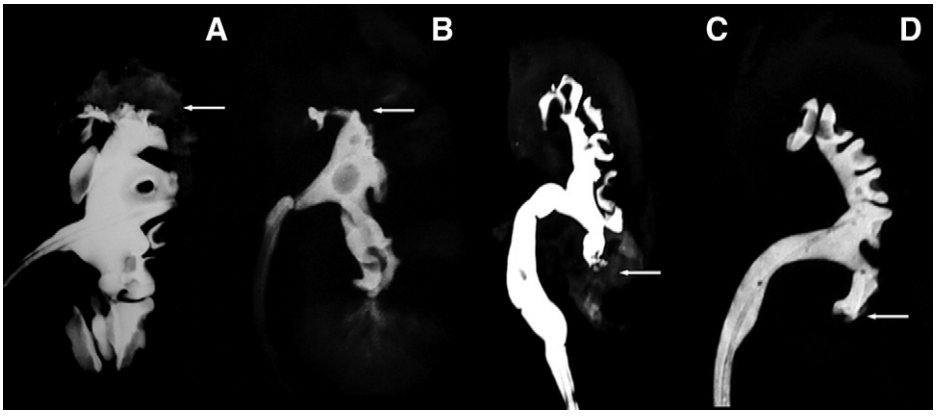

Figure 3. Examples of *ex vivo* retrograde pyelograms performed 14 days after left kidney laparoscopic partial nephrectomy without closure of the collecting system in the cranial pole **(A** and **B)** and in the caudal pole **(C** and **D)**, which shows the absence of contrast medium leakage at the operated poles (arrows).

Under microscopy, the operated pole was always completely covered by connective fibrotic tissue in all analyzed fields (Figure 4). Several adherences between the operated pole and adjacent organs were also observed under microscopy. The connective tissue showed a great density of fibroblasts, which were more commonly seen around the blood vessels. The Sirius red–stained sections, when observed under polarized light, showed that the connective tissue covering the operated pole was bright red, indicating a predominance of type I collagen (Figure 4).

COMMENT

The development of new surgical technologies and their translation from research to the clinical setting require appropriate experimental models. Concerning partial nephrectomy and its modalities (laparoscopic, robotic, natural orifice translumenal endoscopic surgery [NOTES], and single-port), the pig kidney has been the most used model for research and training.[4,7,15,16] Because some methods for collecting system repair have been tested in pigs before clinical use, a deep knowledge of this model for this purpose is necessary.

In the present study, we demonstrated that the pig kidney submitted to a partial laparoscopic nephrectomy of 25% of its parenchymal length, without any attempt to collect system closure or internal drainage, healed with great deposition of collagen and presented with firm adherences to adjacent organs. These observations do not agree with clinical data in human beings, in whom urinary leakage after this kind of surgery is considered a common and major complication,[17] and is observed in 1.9%-5.5% of patients, even after suturing of the collecting system and applying of sealant agents.[6,18] Based on this, we can infer that the pig collecting system healing is quite different from that in human beings.

According to Ames et al.,[12] after partial nephrectomy without suturing the collecting system, the pigs did not develop urinoma, but neither retrograde pyelograms during necropsy nor histologic evaluation of the resection site were performed. These authors also postulated that the absence of Gerota's fascia and diminished renal adipose capsule in pigs would allow urine to flow into the peritoneal cavity and to be absorbed. Nevertheless, the findings of our present study are not in agreement with this theory, as our analysis showed no significant alterations in the levels of urea and creatinine both in serum and in peritoneal fluid.

Also, at the 14th postoperative day, retrograde pyelograms showed a complete sealing of the collecting system; and although infusion pressure was not measured (as performed in other studies[10,19]), the penetration of contrast media into renal parenchyma and rupture of nonoperated pole (Figure 3) indicates that the examination was performed under higher than physiological pressures and that no urine leakage would occur.

It is possible that the small and more muscular collecting system in pigs would seal temporarily the collecting system, allowing collagen deposition without urine leakage. Histologic and quantitative studies supporting this theory could help in further understanding of the collecting system healing in pigs.

The deposition of firm connective tissue over the operated kidney is a common finding in other studies, which applied different methods of hemostasis and collecting system repair after partial nephrectomies in pigs,[4,19-21] although local adherences, as seen in our study, were not reported. The great amount of fibroblasts in tissue examined 14 days after surgery, especially around blood vessels, indicates that synthesis and degradation of collagen may still be occurring.

Recent methods proposed for collecting system closure in preclinical studies that have used the pig as the experimental model should be evaluated carefully. For instance, the use of laser resection, fibrin sealant powder or glue, intracorporeal suture with LapraTy and barbed suture have been described as effective for collecting system closure based on experimental studies in pigs.[7-9,22,23] Analyzing our present findings, one may question the real

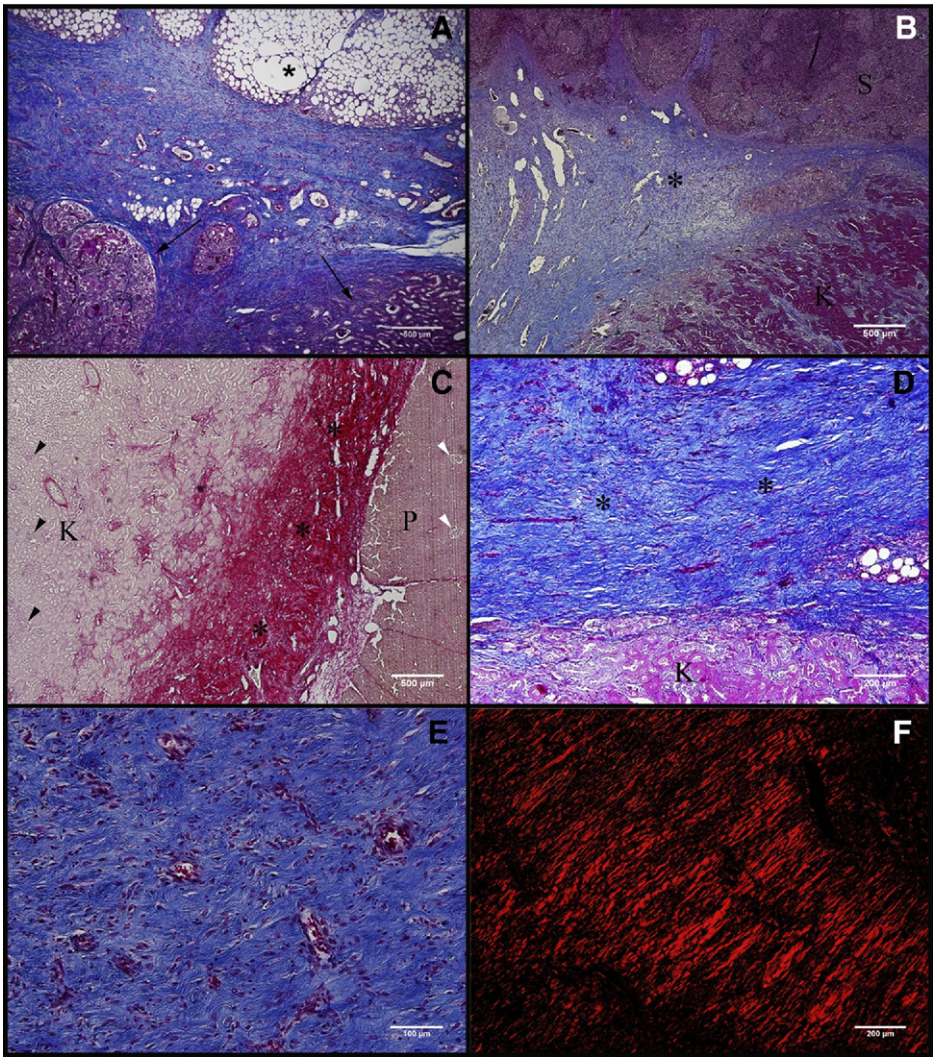

Figure 4. Histologic analysis of the operated region in left kidneys subjected to partial nephrectomy of cranial and caudal poles, on the 14th postoperative day. **(A)** Section of a caudal pole shows **c**onnective tissue between the renal parenchyma (arrows) and the adipose renal capsule (asterisk). Masson's trichrome stain, original magnification ×40. **(B)** Section of a cranial pole demonstrating adherence (asterisk) between splenic tissue (S) and kidney parenchyma (K). Masson's trichrome stain, original magnification ×40. **(C)** Section of a cranial pole showing the pancreas (P) attached to the kidney parenchya (K) by collagen (asterisks). Note islets of Langerhans (white arrowheads) and glomeruli (black arrowheads). Picrosirius red, ×40. **(D)** Section of a caudal pole shows the kidney parenchyma at the operated pole (K) covered by connective tissue (asterisks). Masson's trichrome stain, original magnification ×100. **(E)** Section of a caudal pole shows a strong presence of fibroblasts in the connective tissue. Masson's trichrome stain, original magnification ×200. **(F)** Section of a caudal pole demonstrates a predominance of type I collagen covering the operated renal pole. Picrosirius red under polarization, ×100.

role of these methods, as the pig collecting system heals well without any kind of method for closure or internal drainage.

Other animal models should be assessed for its suitability to kidney collecting system healing studies. In a small experiment with 2 dogs that underwent laparoscopic partial nephrectomy without collecting system closure, the authors reported no urinoma formation and a fibrotic response around the operated pole.[12] Calves were used in studies regarding hemostasis during laparoscopic partial nephrectomy,[24,25] but no published article focusing on bovine kidney healing was found. Also, sheep and rabbits are other species used as surgical models for other purposes. Further studies regarding kidney healing of calves, sheep, and rabbits, with special attention on collecting system, are required. Even considering renal anatomy discrepancies between these species and human beings, the kidney healing may occur in a way more similar than that observed in pigs and dogs.

In conclusion, our findings clearly demonstrated that the pig kidney is not an adequate experimental model for research on which collecting system healing is an important aspect to be considered.

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