

ENHANCED RENAL CRYOABLATION WITH HILAR CLAMPING AND INTRARENAL COOLING IN A PORCINE MODEL

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ABSTRACT

Objectives. To evaluate the effects of renal vascular control and intrarenal cooling on the size of renal lesions attainable with a 3.4-mm cryoprobe.

Methods. Three groups of pigs underwent unilateral laparoscopic renal cryoablation with a 3.4-mm cryoprobe inserted to a depth of 1 cm. An 8-minute double-freeze cycle was used. One week later, an acute contralateral cryolesion was created before killing the animal. In group 1 (n = 6), bilateral cryolesions were created without hilar clamping or intrarenal cooling. In group 2 (n = 6), the cryolesions were created after hilar clamping alone. In group 3 (n = 6), the cryolesions were created after both hilar clamping and application of intrarenal cooling with saline ice-slush infused into the renal pelvis. After nephrectomy, the gross diameters were determined for each cryolesion. The mean diameters of the zones of complete and partial necrosis were determined by histopathologic examination.

Results. In group 3, the cortex cooled from 36.9°C to a mean of 24.8°C. Acutely, no statistically significant difference was found between the lesions produced with clamping alone (37.6 mm) and intrarenal cooling (40.4 mm); however, both were significantly larger than the control cryolesions (28.7 mm). At 1 week, the area of complete necrosis produced with intrarenal cooling (34.3 mm) was significantly larger than the areas of necrosis produced by clamping alone (27.8 mm) or conventional cryoablation (23.9 mm; alpha = 0.05, Tukey's honestly significantly different [HSD] test).

Conclusions. Enhanced cryolesion necrosis was achieved with intrarenal cooling with a 3.4-mm cryoprobe. Intrarenal cooling may be a valuable adjunct to cryoablation in selected cases. *UROLOGY* 63: 1209–1212, 2004. © 2004 Elsevier Inc.

In recent years, increasingly widespread application of sophisticated imaging technologies has led to increased identification of small solid renal lesions.¹ As the natural history of these small, potentially less aggressive neoplasms is not well characterized, and because up to 20% of these lesions may be benign, nephron-sparing surgery has been increasingly advocated as an alternative to standard radical nephrectomy.^{2,3} Recently, open partial nephrectomy has yielded 5-year survival and morbidity comparable to that after radical nephrec-

tomy in patients with small (less than 4 cm), localized, unilateral renal cell carcinoma and a normal contralateral kidney.⁴

In an effort to minimize the morbidity associated with nephron-sparing surgery, laparoscopic renal cryoablation and other minimally invasive ablative techniques are being investigated as alternatives to open extirpative surgery. These minimally invasive approaches are particularly attractive treatment options for elderly patients with multiple comorbidities, patients with solitary kidneys, and patients with a predisposition to multifocal, recurrent tumor formation (ie, von Hippel-Lindau disease). Recently, renal cryoablation has been clinically explored because of the ability to closely monitor the iceball in real time with intraoperative ultrasonography and because of the reliable tissue ablation that has been achieved in animal models.^{5,6}

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However, the utility and practicality of renal cryoablation is limited by the size of the iceball that can be produced by currently available cryoprobes. The reliable treatment of tumors greater than 2.5 cm in diameter currently requires the use of cryoprobes larger than 3.4 mm or multiple probe configurations that may theoretically increase the risk of hemorrhage or increase the complexity of monitoring.^{7,8} We evaluated the ability of intrarenal cooling (retrograde intracavitary ice-cold saline perfusion) and hilar clamping to increase the area of renal necrosis attainable with a single cryoprobe.

MATERIAL AND METHODS

The Washington University School of Medicine Animal Studies Committee granted permission to perform this study. Eighteen domestic pigs weighing 30 to 60 kg were divided into three groups and underwent unilateral laparoscopic renal cryoablation of a lower pole segment followed by recovery. One week later, a contralateral acute lower pole renal cryolesion was created, both kidneys were harvested and placed in 10% formalin, and the animals were killed with intravenous KCl injection. The immediate point of death was included because the acute lesions' borders delineated the extent of the visually apparent iceball. The 1-week follow-up time was chosen because prior experimental studies have shown that 1 week is sufficient for the manifestation of microscopically unequivocal complete coagulative necrosis after cryoablation and the lesion size does not appear to change significantly between 1 and 3 weeks.⁵ Because the purpose of the study was to elucidate the effect of hilar clamping and intrarenal cooling on the size of mature cryolesions, longer follow-up was not considered essential. The temporal progression of cryolesion necrosis and involution has been documented elsewhere.⁵

The minimal and maximal gross surface diameters were measured for each cryolesion. The depth of probe insertion was kept constant and all lesions extended to the collecting system owing to the thin cortex of the pig kidney; thus, the lesion depth was not thought to be a useful parameter for comparison. Two full cross-sections were taken from each cryolesion, and one section was taken from the unablated portion of each kidney for staining with hematoxylin-eosin and evaluation by an expert pathologist (P.H.). The zones of incomplete and complete, unequivocal, coagulative necrosis were closely inspected and defined microscopically. For the 1-week follow-up lesions, the minimal and maximal widths were measured microscopically for the peripheral area of incomplete necrosis, and the mean diameter of the central zone of complete necrosis was calculated as follows for each lesion: $[(\text{minimal} + \text{maximal gross diameter})/2] - (\text{minimal} + \text{maximal width of partial necrosis})$.

Before surgery, each pig was premedicated with telazol 1 mg/kg, xylazine 1 mg/kg, and ketamine 2 mg/kg. After administration of 0.11 mL/kg atropine, the animals were maintained under general endotracheal anesthesia with 1.5% to 2.5% isoflurane. After laparoscopic mobilization of the lower pole of the kidney and isolation of the renal artery and vein, a 3.4-mm Oncura cryoprobe (Oncura Medical, Westbury, NY) was introduced through a small stab wound in the flank directly over the lower pole. All cryolesions were created in the lower pole using an 8-minute double-freeze cycle with a 3.4-mm argon/helium gas cryoprobe (Oncura Medical) inserted perpendicular to the renal surface to a depth of 1 cm. The single-use cryoprobes used in this study did not display probe-tip surface temperatures. However, Oncura has reported that after a successful test, these probes reliably produce a mean nadir tip surface temperature of -140°C (range -135° to -150°C). Omission of these readings is not significant, because the ac-

tual temperature of the parenchyma is more predictive of necrosis, and it varies focally with the rate and duration of cooling, the distance from the probe, and heterogenous tissue characteristics, especially at the periphery of the iceball. The probes were scored at 1 cm to aid in precise placement. Because laparoscopy is the standard approach for human renal cryoablation at our institution and all probes were inserted perpendicular to the lower pole surface to a depth of 1 cm, probe placement was believed to be reasonably precise. An active thaw was only used between freeze cycles when the renal hilum was occluded to minimize the ischemia time. The mixing of active and more traditional passive thaws was not thought to introduce statistically significant error, because recent experimental work has showed no statistically significant difference in the necrosis produced with active and passive thawing.⁹

In group 1, bilateral cryolesions were created without hilar clamping or intrarenal cooling. In group 2, both the renal artery and vein were clamped with either a laparoscopic bulldog or a Satinsky clamp just before the performance of two 8-minute freeze cycles without renal hypothermia. At the end of the second freeze cycle, the clamp was removed, and the lesion was allowed to thaw passively. In group 3, before insufflation, a 35-cm 12F/14F ureteral access sheath (Applied Medical Resources, Rancho Santa Margarita, Calif) was placed into the renal pelvis under fluoroscopic guidance over a guidewire in 5 cases. In 7 cases, a 35-cm 10F/12F access sheath was used because the 12F/14F sheath was unable to be deployed to the level of the ureteropelvic junction owing to the diminutive size of several of the animals' ureters. A 7.1F pigtail catheter (Cook Urological, Spencer, Ind) was then placed in the renal pelvis through the access sheath, and a sidearm adapter (Cook Urological) was secured in the end of the access sheath over the 7.1F catheter. Laparoscopic dissection was then performed as described above, and a 16F thermocouple (Oncura) was placed into the renal cortex to a depth of 5 mm at the midpole. After the renal artery and vein were both clamped, the access sheath was irrigated with a 3-L bag of iced 0.9% saline suspended 60-cm above the kidney.¹⁰ The temperature of the irrigant in the bags was -1.7°C . After 10 minutes of continuous irrigation, two 8-minute freeze cycles were performed in the lower pole. At the end of the second cycle, the vessels were unclamped, and the irrigation was terminated.

RESULTS

No urinary fistulas, urinomas, or hematomas were noted in the animals in any of the three study groups despite consistent extension of the gross lesions into the medulla and collecting system. No qualitative histopathologic differences were noted in the acute or chronic lesions in group 2 or 3.

In group 1, the mean temperature of the renal cortex before cryoablation was 36.9°C and remained unchanged throughout the procedure. Immediately after killing the pigs, the acute cryolesions appeared as roughly circular, sharply demarcated, areas of hemorrhage on the renal surface with a mean diameter of 28.7 mm (range 26 to 31, SD 1.81). These lesions appeared to encompass the extent of the visually apparent iceball and were centered around the probe site. Microscopically, the lesions displayed vascular congestion and hemorrhage, with hyper eosinophilia of the tubules and glomeruli; no discrete areas of necrosis were discernible in any of the acute lesions. At 1 week, the

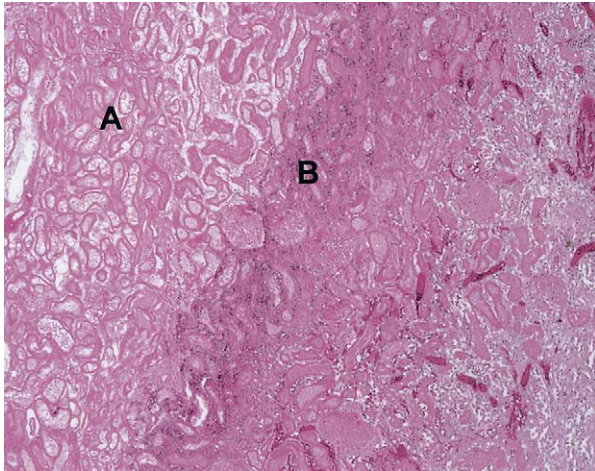


FIGURE 1. Cryolesion periphery at 1 week, with central zone of complete coagulative necrosis (A) and peripheral band of partial necrosis and inflammation (B).

mean gross lesion diameter was 26.9 mm (range 25 to 30, SD 1.68). Microscopically, a central zone of unequivocal, complete coagulative necrosis with a mean diameter of 23.9 mm (range 22.0 to 27.0, SD 1.69) was surrounded by a peripheral rim of incomplete necrosis and inflammation with a mean width of 1.5 mm (range 1.0 to 3.0, SD 0.32; Fig 1).

In group 2, the mean cortical temperature remained 36.8°C at the midpole throughout the procedure. The mean warm ischemia time was 20 minutes, 26 seconds (range 18:53 to 28:20). Acutely, the mean gross lesion diameter was 37.6 mm (range 33 to 41, SD 2.69). At 1 week, the mean gross diameter was 32.5 mm (range 27 to 37, SD 3.69), with a rim of partial necrosis 2.4 mm wide (range 1.0 to 3.5, SD 0.61) encompassing a central zone of complete necrosis 27.8 mm in mean diameter (range 20.0 to 32.5, SD 4.55). No microscopic evidence of ischemic injury in the unablated parenchyma was seen.

In group 3, the mean cortical temperature before hilar clamping was 36.9°C. After intrarenal cooling with iced saline perfusion, the renal cortex cooled to a mean temperature of 24.8°C (range 22° to 30°C). The mean flow of iced saline was 76.7 mL/min (range 65.9 to 91.3) in the seven 10F/12F access sheath trials and was 69.8 mL/min (range 46.9 to 84.5) in the five 12F/14F access sheath trials. No statistically significant differences were observed in the nadir cooling temperatures achieved with the 10F/12F ureteral access sheaths (mean 24.4°C, range 22.0° to 28.0°C) and 12F/14F ureteral access sheaths (mean 25.3°C, range 22.3° to 30.0°C).

The flow rate of the ice-cold saline irrigant was limited by the outflow from the 7.1F pigtail catheter. To some extent, the variability in flow rates and degree of intrarenal cooling may have been a result of subtle differences in the positioning of the access sheath and pigtail catheter within the collecting system. In one trial in which the 12F/14F sheath

TABLE I. Comparison of mean cryolesion dimensions

Size (mm)	Lesion Type*		
	Group 1	Group 2	Group 3
Acute gross diameter	28.7 A	37.6 B	40.4 B
1 week gross diameter	26.9 A	32.5 B	38.3 C
Complete necrosis diameter (1 wk)	23.9 A	27.8 A	34.3 B
Peripheral partial necrosis width (1 wk)	1.5 A	2.4 B	2.0 A,B

* Mean values with different capital letter designations were significantly different statistically ($\alpha = 0.05$).

was positioned slightly below the ureteropelvic junction, a diminished flow rate (46.9 mL/min) was associated with a higher cortical temperature during cooling (30°C). Thus, the trend for higher flow rates noted with the smaller 10F/12F sheath was likely related to the position of the sheath tip. After cryoablation was performed and the vessels were unclamped, the cortex returned to its baseline temperature. The mean cold ischemia time was 29.5 minutes (range 28.6 to 31.5).

Acutely, the mean gross lesion diameter was 40.4 mm (range 37.5 to 45.0, SD 2.67). At 1 week, the mean gross diameter was 38.3 mm (range 35.5 to 40.0, SD 1.81), with an area of partial necrosis 2.0 mm wide (range 1.5 to 4.0, SD 0.50) surrounding a central zone of complete necrosis 34.3 mm in mean diameter (range 32.0 to 36.5, SD 1.84). No evidence of ischemic injury was noted in the surrounding renal parenchyma.

Because our study design compared more than two mean values, an analysis of variance test was used to compare the acute cryolesion diameters, 1-week gross diameters, central complete necrosis diameters, and widths of the peripheral rim of partial necrosis for the three groups (Table I). To control for elevated type I error owing to multiple comparisons, Tukey's Studentized Range (honestly significantly different, HSD) test was used to control the overall type I error at an $\alpha = 0.05$ level. Because this test accounts for both variance within the groups' mean values and sample size, the group mean values with different capital letter designations in Table I were significantly different statistically, despite our small number of trials. Because we did not perform pair-wise two-group comparisons with a *t* test, the *P* values could not be reported for each comparison.

Acutely, the two groups with hilar clamping produced significantly larger iceballs and gross lesions than the standard cryoablation control group; no increase was observed in acute lesion size when intrarenal cooling was added to hilar occlusion. One week after cryoablation, the intrarenal cooling group displayed a significantly larger zone of cen-

tral complete necrosis than the other two groups. Although clamping alone produced grossly larger cryolesions than standard cryoablation, it failed to enlarge the zone of complete necrosis significantly, and it was associated with a significantly larger peripheral rim of incomplete necrosis.

COMMENT

Previous studies have shown that normal porcine renal tissue is completely ablated at temperatures between -16.1° and -19.4°C when puncture cryoablation is performed with modern rapid-freeze cryoprobes.^{11,12} Between this critical temperature and 0°C , varying degrees of cellular injury result, and viable cells may persist. A major advantage of cryoablation over other ablative technologies is that the hyperechoic leading edge of the iceball can be monitored in real time with intraoperative ultrasonography. However, because the temperature at the edge of the iceball is only 0°C and -20°C is only achieved 3.1 mm inside the periphery, the iceball must be extended up to 1 cm beyond the edge of the tumor to ensure complete ablation of the tumor and a margin of normal surrounding tissue.⁷ Furthermore, the critical temperature required to ablate human renal cell cancer in vivo is presently unknown and could be lower than -20°C , reinforcing the need for a generous margin.

Consequently, to treat lesions larger than 2.5 cm reliably, it may be necessary to use either probes larger than 3.4 mm or multiple probe configurations, which may theoretically increase the risk of significant bleeding. A recent study of human renal cryoablation reported a 3000-mL hemorrhage associated with a renal fracture that occurred during the thaw phase of an open procedure using multiple probes.⁸ In that report, using 3-mm and 8-mm probes, an additional four renal capsular fractures occurred that were controlled without the need for transfusion. Even if small 17-gauge probes decrease the risk of bleeding, the placement of multiple probes may increase the complexity of the procedure and may make it more difficult to follow the ablative process reliably with ultrasonography.

A common technique used to increase the volume of necrosis yielded by a single probe is the double freeze-thaw cycle. One recent study noted a significantly increased volume of complete renal necrosis at 4 weeks when a double 15-minute freeze cycle was used instead of a single cycle.⁹ Conversely, although Bishoff and colleagues⁵ reported an increased gross lesion size at 1 week with a double 15-minute freeze, they did not observe an increase in the diameter of complete necrosis. Similarly, Woolley and coworkers⁹ observed no difference in the volume of complete necrosis present at 1 week when active thawing was used instead of the more traditional passive thawing.

A previous animal study showed that renal artery occlusion failed to increase the rate of cooling with cryoablation and concluded that arterial occlusion "provided no significant practical advantage over direct treatment."⁷ In the present study, we noted a significantly increased gross cryoablation diameter with occlusion of both renal artery and vein. Additionally, we observed that the mean diameter of complete central necrosis was 4 mm larger with hilar occlusion alone than it was with conventional cryoablation. Although this difference did not prove statistically significant with this small number of lesions, it could prove statistically significant with a larger study group. Furthermore, intrarenal cooling with hilar clamping produced necrotic cryolesions that were an average of 10 mm larger than standard cryolesions and 6 mm larger than cryolesions with hilar occlusion alone.

CONCLUSIONS

Intrarenal cooling with hilar occlusion may prove to be a useful adjunct to renal cryoablation by facilitating the creation of larger cryolesions.

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