Comparison of Renal Artery, Soft Tissue, and Nerve Damage After Irrigated Versus Nonirrigated Radiofrequency Ablation

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Background—The long-term efficacy of radiofrequency ablation of renal autonomic nerves has been proven in nonrandomized studies. However, long-term safety of the renal artery (RA) is of concern. The aim of our study was to determine if cooling during radiofrequency ablation preserved the RA while allowing equivalent nerve damage.

Methods and Results—A total of 9 swine (18 RAs) were included, and allocated to irrigated radiofrequency (n=6 RAs, temperature setting: 50°C), conventional radiofrequency (n=6 RAs, nonirrigated, temperature setting: 65°C), and high-temperature radiofrequency (n=6 RAs, nonirrigated, temperature setting: 90°C) groups. RAs were harvested at 10 days, serially sectioned from proximal to distal including perirenal tissues and examined after paraffin embedding, and staining with hematoxylin-eosin and Movat pentachrome. RAs and periarterial tissue including nerves were semiquantitatively assessed and scored. A total of 660 histological sections from 18 RAs were histologically examined by light microscopy. Arterial medial injury was significantly less in the irrigated radiofrequency group (depth of medial injury, circumferential involvement, and thinning) than that in the conventional radiofrequency group (P<0.001 for circumference; P=0.003 for thinning). Severe collagen damage such as denatured collagen was also significantly less in the irrigated compared with the conventional radiofrequency group (P<0.001). Nerve damage although not statistically different between the irrigated radiofrequency group and conventional radiofrequency group (P=0.36), there was a trend toward less nerve damage in the irrigated compared with conventional. Compared to conventional radiofrequency, circumferential medial damage in highest-temperature nonirrigated radiofrequency group was significantly greater (P<0.001).

Conclusions—Saline irrigation significantly reduces arterial and periarterial tissue damage during radiofrequency ablation, and there is a trend toward less nerve damage. (Circ Cardiovasc Interv. 2015;8:e001720. DOI: 10.1161/CIRCINTERVENTIONS.114.001720.)

Key Words: irrigation ■ pathology ■ preclinical evaluation ■ radiofrequency catheter ablation

To date, arterial hypertension remains a significant public health concern contributing substantially to global cardiovascular mortality. The estimated total number of adults with hypertension in 2000 was 978 million, and the number of adults with hypertension in 2025 is predicted to increase to 1.56 billion. The association between hypertension and increased death rates has been well established. Although several classes of antihypertensive medications are available for the treatment of hypertension, approximately half of these patients remain uncontrolled. Moreover, ≥15% of all hypertensive patients have resistant hypertension, and fail to achieve blood pressure goals, despite 3 or more antihypertensive medications.

Because the renal sympathetic nervous system is considered to be essential for the development of arterial hypertension, renal sympathetic denervation using catheter-based radiofrequency ablation is a promising new option for patients suffering resistant hypertension. However, the arterial wall injury induced by radiofrequency energy remains a concern, because radiofrequency has been shown to acutely induce luminal thrombus deposition and late stenosis of the renal artery (RA). The concept of saline irrigation has successfully been used during radiofrequency ablation of atrial fibrillation, and macroreentrant atrial and scar-related ventricular tachycardia. Because irrigation has been demonstrated to control surface temperature at the ablation site, it is reasonable to assume that it may also reduce the radiofrequency-induced arterial wall damage during radiofrequency renal sympathetic denervation. The aim of this study was, therefore, to determine if saline irrigation during radiofrequency ablation preserved the RA while allowing effective denervation in a porcine model.
WHAT IS KNOWN

- Renal sympathetic denervation using catheter-based radiofrequency ablation is a promising new option for patients suffering resistant hypertension.
- Arterial wall injury induced by radiofrequency energy remains a concern because radiofrequency has been shown to acutely induce luminal thrombus deposition and late stenosis of the renal artery.

WHAT THE STUDY ADDS

- In a preclinical animal study, saline irrigation during radiofrequency ablation reduced damage to the renal artery media as compared with conventional radiofrequency ablation.
- There was no difference in nerve injury between the saline-irrigated radiofrequency and conventional radiofrequency methods, however; the study revealed trends toward less nerve injury and lower norepinephrine levels with irrigated as compared with non-irrigated ablation.

Methods

Animals

The study was carried out in 9 Yorkshire pigs (44.6–52.6 kg). All RAs were bilaterally treated using a helical-shaped multielectrode (5 radiofrequency emissions) radiofrequency ablation catheter with a multichannel ablation system (RenLane Renal Denervation Catheter; Cordis, Inc, Fremont, CA) with different temperature set point and with/without saline irrigation. The radiofrequency energy limit was set to 15 W at the initiation of the experiment and duration of ablation to 30 s in all groups. The temperature set points regulate power output when maximum temperature at the electrode sensor is reached, which results in a decrease in power to maintain the temperature. Three animals (6 RAs) were assigned to a temperature set point of 50°C with saline irrigation (the irrigated radiofrequency group [n=6]); 3 animals (6 RAs) to a temperature set point of 65°C without saline irrigation (the conventional radiofrequency group [n=6]); and 3 animals (6 RAs) to a temperature set point of 90°C without saline irrigation (the high-temperature radiofrequency group [n=6]). In the irrigated radiofrequency group, irrigation flow rate was 30 mL/min during radiofrequency ablation and 2 mL/min at baseline. Also, in the irrigated radiofrequency group, the power and duration were identical to the conventional radiofrequency group, but because of the irrigation, the temperature set point was reduced to 50°C. All animal procedures were performed at CBSET, Inc (Lexington, MA) after approval of the Institutional Animal Care and Use committee. All procedures and conditions of testing in this study were in compliance with the US Department of Agriculture’s and Animal Welfare Act/Regulations (9 Code of Federal Regulations Parts 1, 2, and 3), following the Guide for the Care and Use of Laboratory animals.

Interventional Procedure

Under fluoroscopic guidance, a sheath was placed in the femoral artery and a guide catheter was advanced to the RAs. Intravenous heparin (50–200 U/kg) and nitroglycerin (200–400 μg, IA) were administered before angiography. The radiofrequency catheter system was introduced through the guide catheter and positioned in each RA for radiofrequency treatment (Figure 1). The radiofrequency catheter system monitored radiofrequency impedance and temperature during radiofrequency ablation for each electrode throughout the course of the treatment. The RA diameter ranged from 4.6 to 6.6 mm, and the RA length ranged from 14 to 50 mm. Radiofrequency ablation was performed from the RA ostium to the bifurcation in 15 vessels, and from the RA ostium to the middle segment of the RA in 3 vessels; if electrodes protruded into the RA bifurcation, they were turned off. Similar treatment was also performed in the contralateral RA. Aspirin (650 mg) and clopidogrel (300 mg) were administered on day 1, and aspirin (81 mg) and clopidogrel (75 mg) were administered daily thereafter for the remainder of the study. All animals were euthanized at 10 days. The RAs were perfusion-fixed at 100 mm Hg with 10% neutral-buffered formalin for ≈20 to 30 minutes. The aorta, RA with intact surrounding soft tissues and kidneys were removed for further histopathologic assessment.

Tissue Dissection and Paraffin Embedding

Each treated RA was sequentially cut from proximal to distal into 7 to 9 segments, followed by dehydration and paraffin embedding. Each paraffin block was serially sectioned at 500 μm intervals ≤2000 μm, (a total of 5 sections examined per block) and stained with hematoxylin-eosin and modified Movat pentachrome, whereas adjacent unstained slides were reserved for Masson trichrome and immunohistochemical staining.

Histological Assessment

To assess treatment effects on the RA, adjacent nerve fascicles, arterioles, vein, and soft tissue, ordinal data were collected and semiquantitated using an ordinal grading scheme of 0 to 4: 0=none, 1= minimal, 2=mild, 3=moderate, and 4=severe. Maximal nerve damage was recorded in each section. Perineuronal injury such as perineuronal inflammation or fibrosis and endoneuronal injury, such as vacuolization, digestion chambers, pyknotic nuclei, and necrosis were evaluated. Vacuolization was defined by the presence of vacuolated areas, containing loose strands of tissue interspersed with areas of homogeneous eosinophilic staining of cell cytoplasm and nuclei showing...
pyknosis (compressed and deformed nuclei). Digestion chambers were characterized by the presence of aggregated myeline (eosinophilic hyaline globules) and vacuolization at the periphery of the cell with or without vesicular nuclei interspersed.

RA and Vein Injury
Endothelium damage was circumferentially evaluated: 0=no endothelial loss, 1=endothelial loss <25% of vessel circumference, 2=endothelial loss 25% to 50% of vessel circumference, 3=endothelial loss 51% to 75% of vessel circumference, 4=endothelial loss >75% of vessel circumference. Media injury was evaluated both by the depth and circumference of involvement separately: 0=no media change, grade 1=medial injury involving <25% of media depth/circumference, grade 2=medial injury 25% to 50% of media depth/circumference, grade 3=medial injury 51% to 75% of medial depth/circumference, and grade 4=medial injury >75% of medial depth/circumference. Furthermore, the presence of arterial medial thinning was also evaluated, and was defined as thickness of media at the site of damage (mm/unaffected media thickness (mm)<0.5. These changes were the result of severe smooth muscle cell loss/necrosis within the media.

Arterioles and Surrounding Soft Tissue Injury
Arterial injury was also semiquantitated using a grading system of 0 to 4, which was applied with respect to inflammation/fibroinoid necrosis: 0=none, 1=minimal, 2=mild, 3=moderate, and 4=severe. Prevalence of denatured collagen, which seems as basophilic staining of collagenous tissue from thermal injury, was assessed as absent (0) or present (1). Distance from arterial lumen to deepest soft tissue damage and ablation area were measured in each RA using morphometric software (IP Laboratory for Mac OS X, Scanalytics, Rockville, MD).

Quadrant Analysis
To evaluate the extent of tissue damage circumferentially, the following parameters were evaluated: (1) Number of quadrants with nerve fascicles, (2) Number of quadrants with injured nerve fascicles (grade ≥2). (3) Number of quadrants with moderate to severely injured nerve fascicles (grade ≥3). (4) Number of quadrants with injured periarterial soft tissue (collagen and fat necrosis).

Immunostaining of Perivascular Nerves and Scoring Criteria
The extent of structural and functional nerve injury was also assessed after immunostaining against S-100 protein (dilution 1: 4000; Dako, Carpinteria, CA) for the recognition of axons within nerve fascicles and against tyrosine hydroxylase (TH; 1: 100; EMD Millipore, Billerica, MA), an important enzyme required for the synthesis of norepinephrine. S-100 was used for the recognition of nerve fascicles, whereas TH was used as an indicator of functional nerve damage. The intensity and distribution of staining was semiquantified using a scoring scheme of 0 to 3: 0=no reaction, 1=very weak or patchy reaction, 2=weak reaction, and 3=strong reaction. Minimum score was recorded in each selected section.

Renal Tissue Norepinephrine Assay
Norepinephrine levels in porcine kidney tissue were measured by high-performance liquid chromatography-mass spectrometry assay as previously described.

Statistical Analysis
Results for continuous variables were expressed as mean±SD, whereas results for score data were expressed as median and interquartile range (IQR). Normality of distribution was tested with the Wilk–Shapiro test. Statistical comparisons of normally distributed measurements were performed by linear generalized estimating equation modeling with an assumed Gaussian distribution, an identity link function, and an assumed exchangeable structure for the within-cluster correlation matrix in consideration of the clustered nature of ≥1 individual measurements from 1 animal. For the comparison of non-normally distributed data, Mann–Whitney U test was used. Direct comparisons between the irrigated radiofrequency versus conventional radiofrequency and between conventional radiofrequency versus high-temperature radiofrequency were performed with Bonferroni adjustment. All tests were 2-tailed, and the analyses with Bonferroni adjustment required P<0.025 for statistical significance. The conventional radiofrequency group was considered to be the control group. The Spearman correlation coefficient was calculated to assess the correlation between norepinephrine levels and semiquantitative histopathologic scores. All analyses were performed with SPSS software (version 19; Chicago, IL) and JMP 5 (SAS Institute, Cary, NC).

Results
All 9 animals survived the expected-in-life phase of the study. A total of 660 sections were reviewed from 18 treated RAs. Of 660 sections, 253 sections from post bifurcation sections (untreated site) were excluded from analysis. A total of 407 sections (22.6 sections/RA) from 18 RAs were used for detailed analysis, and mean values were calculated for each RA treated. Study flow chart is shown in Figure 2.

Irrigated Radiofrequency Versus Conventional Radiofrequency
During treatment, 1 RA (17%) showed vasospasm in the conventional radiofrequency group, whereas there was no vasospasm in the irrigated radiofrequency group. The comparison of arterial and periarterial soft tissue injury is shown in Table 1. Arterial endothelial loss was not observed in both groups. Arterial media depth of injury was less in the irrigated radiofrequency group (3.1 [IQR, 2.5–3.5]) than that observed in the conventional radiofrequency group (3.5 [IQR, 3.1–4.0]) without reaching statistical significance (P=0.05). Arterial media circumferential injury was significantly less in the irrigated radiofrequency group (1.1 [IQR, 0.8–1.4]) than that in the conventional radiofrequency group (1.9 [IQR, 1.5–2.4]; P=0.001). Prevalence of arterial media thinning, which represents severe medial damage was also significantly less in the irrigated radiofrequency group (0.3 [IQR, 0–0.6]) compared with the conventional radiofrequency group (0.8 [IQR, 0.6–1.0]; P=0.003). Representative arterial serial sections from each group are shown in Figure 3.

Figure 2. Study flow chart.
Renal veins were well preserved in both groups. Arteriolar injury in the irrigated radiofrequency group (2.8 [IQR, 2.1–3.4]) was significantly less than that in the conventional radiofrequency group (3.3 [IQR, 2.6–4.0]; \(P=0.02\)). Prevalence of denatured collagen, which represents severe soft tissue damage, was significantly less in the irrigated radiofrequency group (0 [IQR, 0–0.2]) compared with the conventional radiofrequency group (0.4 [IQR, 0.3–0.6]; \(P<0.001\)). Distance from arterial lumen to deepest tissue injury was shorter in the irrigated radiofrequency group (4.7±1.3 mm) than that in the conventional radiofrequency group (5.3±0.9 mm) without reaching statistical significance (\(P=0.04\)). The number of quadrants of injured soft tissue was significantly less in the irrigated radiofrequency group (1.0±0.3) than that in the conventional radiofrequency group (1.5±0.4; \(P=0.02\)).

Comparison of nerve damage and representative images are shown in Table 2 and Figure 4, respectively. Nerve injury score was similar among the irrigated radiofrequency (2.5 [IQR, 2.1–3.2]) and the conventional radiofrequency groups (2.8 [IQR, 2.6–2.9]; \(P=0.36\)). Injured nerve (score \(\geq 2\)) quadrants/total quadrants with nerves, which represents an assessment of circumferential nerve damage, although less in the irrigated radiofrequency group (0.6±0.1) compared with the conventional radiofrequency group (0.7±0.1), did not reach statistical significance (\(P=0.09\)). Moderate to severe nerve injury (score \(\geq 3\)) quadrants/total quadrants with nerves was significantly less in the irrigated group (0.2±0.1) than that in the conventional radiofrequency group (0.4±0.1; \(P=0.007\)). TH score was numerically greater in the irrigated radiofrequency group (1.6 [IQR, 1.1–2.0]) compared with the conventional radiofrequency group (1.2 [IQR, 1.0–1.5]; \(P=0.33\)). Representative sections from the irrigated and conventional groups are shown in Figure 5. Mean norepinephrine concentration was also numerically greater in the irrigated radiofrequency group (280±214 ng/g) compared with the conventional radiofrequency group (202±215 ng/g; \(P=0.48\)).

### Table 1. Comparison of Arterial and Periarterial Soft Tissue Damage Between the Irrigated Radiofrequency, Conventional Radiofrequency, and High-Temperature Radiofrequency Groups

<table>
<thead>
<tr>
<th>Damage</th>
<th>Irrigated Radiofrequency Group (n=6)</th>
<th>Conventional Radiofrequency Group (n=6)</th>
<th>High-Temperature Radiofrequency Group (n=6)</th>
<th>(P) Value for Irrigated vs Conventional Radiofrequency</th>
<th>(P) Value for Conventional vs High-Temperature Radiofrequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial endothelial loss (score 0–4)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0.2 (0–0.7)</td>
<td>...</td>
<td>0.02</td>
</tr>
<tr>
<td>Arterial media depth injury (score 0–4)</td>
<td>3.1 (2.5–3.5)</td>
<td>3.5 (3.1–4.0)</td>
<td>4 (3.5–4)</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>Arterial media thinning (score 0–1)</td>
<td>0.3 (0–0.6)</td>
<td>0.8 (0.6–1.0)</td>
<td>0.9 (0.8–1.0)</td>
<td>0.003</td>
<td>0.23</td>
</tr>
<tr>
<td>Arterial media circumferential injury (score 0–4)</td>
<td>1.1 (0.8–1.4)</td>
<td>1.9 (1.5–2.4)</td>
<td>3.4 (2.7–3.8)</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Venous endothelial loss (score 0–4)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0.01)</td>
<td>...</td>
<td>0.32</td>
</tr>
<tr>
<td>Venous media depth injury (score 0–4)</td>
<td>0 (0–0.03)</td>
<td>0 (0–0.06)</td>
<td>0.5 (0–1.0)</td>
<td>0.90</td>
<td>0.05</td>
</tr>
<tr>
<td>Venous media circumferential injury (score 0–4)</td>
<td>0 (0–0.01)</td>
<td>0 (0–0.06)</td>
<td>0.1 (0–0.2)</td>
<td>0.90</td>
<td>0.11</td>
</tr>
<tr>
<td>Arterioles injury (score 0–4)</td>
<td>2.8 (2.1–3.4)</td>
<td>3.3 (2.6–4.0)</td>
<td>3.6 (3.2–4)</td>
<td>0.02</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Denatured collagen (score 0–1)</td>
<td>0 (0–0.2)</td>
<td>0.4 (0.3–0.6)</td>
<td>0.9 (0.8–1.0)</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Distance to deepest tissue injury, mm</td>
<td>4.7±1.3</td>
<td>5.3±0.9</td>
<td>7.2±2.6</td>
<td>0.04</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Number of quadrants of injured soft tissue</td>
<td>1.0±0.3</td>
<td>1.5±0.4</td>
<td>2.5±0.7</td>
<td>0.02</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Ablation area, mm²</td>
<td>14.2±10.1</td>
<td>28.0±10.0</td>
<td>47.5±22.2</td>
<td>0.06</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Median (Q1–Q3) or mean±SD.

High-Temperature Versus Conventional Radiofrequency Ablation

Five RAs (83% of 6 RAs) showed vasospasm during treatment in the high-temperature radiofrequency group. Arterial endothelial damage was only observed in the high-temperature radiofrequency group. Although arterial media depth or prevalence of media thinning were not statistically different in the high-temperature radiofrequency group compared with the conventional radiofrequency group, arterial media circumferential damage was far more extensive in the high-temperature radiofrequency group (3.4 [IQR, 2.7–3.8]) than that in the conventional radiofrequency group (\(P<0.001\)). Similarly, arteriolar damage (3.6 [IQR, 3.2–4.0]), presence of denatured collagen (0.9 [IQR, 0.8–1.0]), distance to deepest tissue injury (7.2±2.6), and number of quadrants...
Comparison of Irrigated vs Nonirrigated Radiofrequency Ablation

Correlation Between Norepinephrine Concentration and Semiquantitative Scoring

To investigate the relationship between kidney tissue concentration of norepinephrine and semiquantitative scoring of nerve injury, Spearman’s correlation efficient was used. Norepinephrine concentration was significantly correlated with nerve injury score (Spearman’s $r=-0.49$; $P=0.04$), TH score (Spearman’s $r=-0.69$; $P=0.002$), injured nerve (score $\geq 2$) quadrants/number of nerve quadrants (Spearman’s $r=-0.56$; $P=0.02$), and moderate to severe nerve injury (score $\geq 3$) quadrants/number of nerve quadrants (Spearman’s $r=-0.61$; $P=0.008$; Figure I in the Data Supplement).

Discussion

The present histopathologic study aimed to assess the histopathologic effects of saline-irrigated radiofrequency ablation on periarterial renal nerve and vascular/perivascular tissue injury in comparison with conventional nonirrigated application of radiofrequency energy. Compared to conventional radiofrequency ablation, saline irrigation during radiofrequency ablation not only significantly reduced the extent of arterial damage but also arteriolar and soft tissue (collagen and fat) damage. Despite a trend toward numerically lower overall nerve injury, there was no statistical difference observed between the saline-irrigated and conventional radiofrequency groups, whereas maximum nerve injury (score $\geq 3$) by quadrant analysis was significantly greater in the conventional nonirrigated group.

Radiofrequency application to ablate periarterial sympathetic nerves has recently been introduced as a viable option to treat patients suffering treatment-resistant arterial hypertension. Although the main mechanism of this novel treatment modality is thought to result from morphological and
functional disruption of neuronal cross-talk among sympathetic and sensory nerves accompanying the periarterial tissue, this benefit may be achieved at the collateral cost of transmural arterial injury potentially resulting in long-term sequelae such as RA stenosis. With the introduction of saline irrigation during radiofrequency ablation of atrial fibrillation, a tremendous reduction in tissue damage was reported while preserving the spatial and circumferential ablation zone.20 Therefore, the main purpose of using saline irrigation for renal radiofrequency denervation is to protect renal arterial damage by controlling surface temperature at the ablation site.13,14 Our results suggest that such success can be achieved without significant reduction of nerve damage. Another likely effect could be that saline irrigation could expand ablation area. In this regard, Nakagawa et al reported that total ablation area by saline-irrigated radiofrequency ablation was significantly larger compared with nonirrigated radiofrequency ablation in a dog’s thigh muscle model. In their study, saline irrigation maintained a low electrode–tissue interface temperature during radiofrequency application at high power, which prevented an impedance rise and produced deeper and larger lesions.21 However, our results suggest that saline irrigation reduced ablation zone. Differences in target tissue and energy setting could partly explain the opposite results that were observed in our study. From our point of view, controlling the ablation zone is more important than expanding it, as excess energy delivery may result in injury of the visceral organs such as the small intestine.

**How Does Saline Irrigation Affect RA and Surrounding Tissues?**

Although our results suggest that saline irrigation reduced both axial and circumferential radiofrequency ablation zone, the effect on circumferential reduction was more pronounced. Median arterial medial circumferential injury score in the irrigated radiofrequency group was 1.1 (IQR, 0.8–1.4), which means that ≈25% of cross-sectional RA area was injured, whereas median arterial media circumferential injury score in the conventional radiofrequency group was 1.9 (IQR, 1.5–2.4), which suggests that ≈50% of RA area was injured. Because long-term safety of the RA is of utmost importance in

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**Table 2. Comparison of Nerve Damage Between the Irrigated Radiofrequency, Conventional Radiofrequency, and High-Temperature Radiofrequency Groups**

<table>
<thead>
<tr>
<th></th>
<th>Irrigated Radiofrequency Group (n=6)</th>
<th>Conventional Radiofrequency Group (n=6)</th>
<th>High-Temperature Radiofrequency Group (n=6)</th>
<th>P Value for Irrigated Radiofrequency vs Conventional Radiofrequency</th>
<th>P Value for Conventional Radiofrequency vs High-Temperature Radiofrequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve injury (score 0–4)</td>
<td>2.5 (2.1–3.2)</td>
<td>2.8 (2.6–2.9)</td>
<td>3.5 (3.2–3.8)</td>
<td>0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Injured nerve (score ≥2) quadrants/number of nerve quadrants</td>
<td>0.6±0.1</td>
<td>0.7±0.1</td>
<td>0.9±0.1</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Moderate to severe injured nerve (score ≥3) quadrants/number of nerve quadrants</td>
<td>0.2±0.1</td>
<td>0.4±0.1</td>
<td>0.7±0.2</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyrosine hydroxylase (score 0–3)</td>
<td>1.6 (1.1–2.0)</td>
<td>1.2 (1.0–1.5)</td>
<td>0.8 (0.6–1.3)</td>
<td>0.33</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean norepinephrine concentration (ng/g)</td>
<td>280±214</td>
<td>202±215</td>
<td>37±67</td>
<td>0.48</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Median (Q1–Q3) or mean±SD.

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**Figure 4.** Representative injured nerve images from irrigated radiofrequency (RF) and conventional RF groups. Perineural fibrosis is observed in Movat and hematoxylin-eosin (H&E) stain. S-100 stain (pan nerve marker) confirms nerve architecture. Weak reaction to tyrosine hydroxylase suggests functional nerve damage.
renal sympathetic denervation procedures, substantial reduction of arterial media injury is the most important benefit arising from saline irrigation.

Mean number of quadrants of injured soft tissue in the irrigated radiofrequency group was approximately two thirds of that in the conventional radiofrequency. There are several organs, such as renal vein, arterioles, lymph nodes, fat, and ureters around RA. When the ablation energy involves those organs, it is unclear whether these ablated organs will provoke adverse outcomes in the future. Saline irrigation should help preserve these organs keeping them free of injury.

Although overall nerve injury in the irrigated radiofrequency group was not statistically different from that in the conventional radiofrequency, moderate to severe injured nerve quadrants/total nerve quadrants was less in the irrigated radiofrequency group at each site than that in the conventional radiofrequency group, suggesting the possibility that total number of denervated nerves is less in irradiation radiofrequency as compared with nonirrigation radiofrequency ablation. As a critical damage threshold to predict efficacy in decreasing blood pressure in clinical trials cannot be extrapolated from the current animal study, dedicated clinical trials are warranted to delineate the relative merits of this technology. However, the evaluation of nerve injury is based on a single ablation site, therefore, we cannot conclude that the RenLane device does not achieve circumferential ablation because it delivers radiofrequency energy at ≤5 different sites simultaneously in a single treatment setting. This study provides meaningful insights into the correlation of kidney norepinephrine levels and nerve injury assessed by histopathology and immunohistochemistry. Although the positive correlation of these parameters suggest predictive value in determining efficacy of sympathetic denervation in preclinical animal models, a causative association with reduction of blood pressure in man cannot be established in our study.

**Clinical Implications**

Although clinical trials report a reduction of blood pressure ≤3 years after radiofrequency denervation, studies about long-term RA safety remain scarce. Templin et al. reported RA wall damage by radiofrequency-catheter ablation using optical coherence tomography. Endothelial-intimal edema and thrombus formation were observed in 96% and in 67% of cases, respectively. Because RA stenosis in the chronic phase and RA intimal edema and thrombus in the acute phase have been reported by conventional radiofrequency ablation, protecting the RA from radiofrequency energy becomes increasingly important. In light of the most recent findings in clinical trials investigating the effect of renal denervation therapy in patients with resistant hypertension in a randomized and sham-controlled fashion, experimental preclinical studies such as the current one are strongly warranted to help refine technological standards.

**Study Limitations**

First, although the anatomy of renal arteries in pigs is similar to that in human, we should exercise caution when translating preclinical results to human disease condition. Despite the fact that histopathologic assessment of periarterial renal nerves provides a fragmented view on overall nerve anatomy, step sectioning of histopathologic samples every of 500 μm allowed us to investigate each vessel in a more comprehensive manner than what is conventionally done. As histopathologic data collection was not performed in a blinded manner, there is a chance of bias during histopathologic scoring. However, we have recently reported a histological systematic scoring scheme for the assessment of preclinical renal denervation, and we have used the same scheme in this study. Moreover, excellent correlation was observed between norepinephrine levels and histological semiquantitative nerve injury scores as demonstrated in this study. Although nerve injury score, TH score, and norepinephrine concentrations among the irrigated and conventional radiofrequency groups were not statistically different, there is a possibility of β error because of small sample size that may have resulted in failure to detect statistically significant differences. However, the methodological constraints, which lead to examination of nerve injury in single cross-sectional planes versus verification of the three-dimensional reconstruction of radiofrequency injury at 5 different locations could likewise have resulted in underestimation of renal denervation in the irrigated radiofrequency group. In addition, the absence of control norepinephrine levels of native kidney tissue implies an important limitation of this study as
the comparative magnitude of sympathetic denervation among treatment groups cannot be appreciated. In each group, the temperature set points regulate power output when maximum temperature at the electrode sensor is reached, however, real-time feedback of tissue temperature is not provided in the current generation of device, which can be a confounding factor for the treatment effect. The reason of a decreased temperature set point (50°C) in the irrigated ablation group can be seen in providing an additional safety margin because the temperature at the electrode sensor may underestimate the temperature achieved in arterial tissue and remains a clinical standard. Saline irrigation was linked to a temperature set point of 50°C to provide an appropriate safety margin, which makes it difficult to distinguish the effect of saline irrigation from the effect of temperature. Because our study was designed to evaluate subacute effects on RA and nerve injury, a follow-up of 10 days was chosen, however, longer-term follow-up is needed to determine chronic effects of this technology.

Conclusions
Compared to conventional radiofrequency, saline irrigation during radiofrequency ablation reduced arterial media damage of the RA. Saline irrigation also reduced periarterial soft tissue damage. Nerve injury was statistically not significantly different between the saline-irrigated radiofrequency and conventional radiofrequency, however, the study revealed trends toward less nerve injury and norepinephrine levels with irrigated as compared with nonirrigated ablation. From this study, it can be concluded that saline irrigation during renal sympathetic denervation using the currently applied settings results in improved preservation of vascular and perivascular tissue, and, therefore, the net therapeutic effect with respect to balancing safety and efficacy seems to be equivalent. However, moderate to severe injured nerve (score ≥ 3) quadrants/number of nerve quadrants was less in the irrigated versus conventional radiofrequency groups. Because nerve injury and norepinephrine levels cannot be directly equated to more or less effective reduction of blood pressure in our animal model, these subtle differences will need to be critically scrutinized in dedicated clinical trials in man.

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References
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Supplemental Figure 1. Correlation between NEPI concentration and semi-quantitative scoring

**Figure Legends.**

*A*: Correlation between NEPI concentration and nerve injury score (0-4).  
*B*: Correlation between NEPI concentration and Tyrosine Hydroxylase score (0-3).  
*C*: Correlation between NEPI concentration and injured nerve (score 2 or greater) quadrants/number of nerve quadrants.  
*D*: Correlation between NEPI concentration and moderate to severe injured nerve (score 3 or greater) quadrants/number of nerve quadrants.