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Comparison of branch and distally focused main renal artery denervation using two different radio-frequency systems in a porcine model

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ABSTRACT

Objectives: Anatomic placement of lesions may impact efficacy of radio-frequency (RF) catheter renal denervation (RDN). However, it is unclear if it is necessary to perform treatments post bifurcation with systems that may provide deeper penetration to achieve successful RDN.

Methods: Sixteen domestic swine ($n = 16$) were randomly assigned to 4 groups: 1) 8 lesions created in the branch arteries using the Spyral catheter (SP8); 2) 8 lesions created in the branch arteries plus 4 lesions created in the main artery using the SP catheter (SP12); 3) 8 lesions created in the main artery using the EnligHTN catheter with the distal position as close as possible to the bifurcation (EN8); and 4) 12 lesions created in the main artery using the EN catheter with the distal position as close as possible to the bifurcation (EN12).

Results: Each arm showed statistically significant changes in kidney norepinephrine (NE, ng/g) between treated kidneys vs. untreated contralateral control. There were no statistically significant differences in tissue NE% reductions across each arm based on catheter, anatomic location, & number of lesions ($p = 0.563$): **EN8** −74 ± 34%, **EN12** −95 ± 3%, **SP8** −76 ± 16%, **SP12** −82 ± 17% ($p = 0.496$). A total of 46 lesions were measured for lesion depth: EN main (3.3 ± 2.8 mm) vs. SP branch (2.0 ± 1.0 mm, $p = 0.039$), SP main (2.9 ± 1.6 mm) vs. SP branch ($p = 0.052$), and EN main vs. SP main ($p = 0.337$).

Conclusions: Distally-focused main renal artery treatment using the EN system appears to be equally efficacious in reducing tissue NE levels compared with SP treatment in the branches plus main renal arteries, advocating for device-specific procedure execution.

1. Introduction

Renal artery radio-frequency (RF) ablation (RDN) is under investigation for the treatment of hypertension [1]. Recent preclinical studies evaluating lesion locations in the distal vs. proximal renal arteries and innervation pattern provided important information on the optimal target treatment location [2–7]. Sympathetic nerves are more accessible to RF therapy distally and post-bifurcation versus proximally in the main renal artery because of their closer proximity to the renal arterial lumen [2]. In a porcine model, RF energy delivery to the branches and the main renal artery resulted in less variability and greater reduction of both NE and axon density compared to conventional treatment of the main renal artery alone. [7]. However, not all existing catheter systems were developed for treatment of smaller branch arteries and some catheters are not capable to enter smaller branch arteries safely. Conversely, it is unclear if it is necessary to perform treatments post bifurcation to achieve successful RDN. Limited efficacy and safety evidence exist comparing treatments with the EN catheter focused distally in the main renal artery versus the SP catheter when targeted in the branch arteries or branch arteries plus main renal artery. The present study was designed to investigate the overall efficacy of two RF RDN systems and to correlate lesion formation by histopathology with bioanalytical markers of RDN treatment success within the main

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renal artery and/or bifurcations before embarking on a pivotal clinical study.

2. Methods and materials

2.1. Study design

Sixteen domestic swine (45 to 55 kg) were anesthetized, vascular access acquired via the femoral artery, and pre-treatment angiography of the renal arteries performed to identify potential treatment sites and screen for accessory arteries. Main and branch renal arterial segments were measured, including overall length from the bifurcation to the ostium, as well as the minimum, maximum, and mean diameter. Blood pressure and heart rate were monitored continuously and sterility was maintained throughout the procedure. Blood urea nitrogen (BUN), creatinine, and urine protein/creatinine ratio were measured at baseline and termination to assess renal function (Marshfield Laboratories, Marshfield, WI, USA). All animals post-term had immediate kidney explantation for bioanalytical analysis of norepinephrine content. All kidneys were placed in liquid nitrogen within 3 min from time of death. Kidney NE measurements were taken from homogenized whole kidneys using a validated test method (validated as ≤3% standard deviation). The procedures were performed in accordance with institutional guidelines. In the event the generator did not complete a full therapy cycle the catheter was repositioned, treatment was repeated, and the original lesions were not included in the final count.

2.2. Ablation procedure

Animals were randomly assigned to 4 treatment groups: 1) 8 lesions created in the branch arteries using the SP catheter (SP8); 2) 8 lesions created in the branch arteries plus 4 lesions created in the main artery using the SP catheter (SP12); 3) 8 lesions created in the main artery using the EN catheter with the distal position as close as possible to the bifurcation (EN8); and 4) 12 lesions created in the main artery using the EN catheter with the distal position as close as possible to the bifurcation (EN12). RF ablation lesions were created in the left or right main renal artery using either the EN 8 French (F) compatible, multi-electrode, expandable, basket RDN catheter and generator (EN, St. Jude Medical, St. Paul, MN) or the 6-F compatible SP catheter and generator (SP, Medtronic PLC, Dublin, Ireland). Both systems use an indifferent skin patch and deliver RF energy from four electrodes in a unipolar, simultaneous fashion. Either the small or large basket EN catheter (for mean vessel diameters of 4–6 mm and 5.5–8 mm, respectively) was delivered to the target artery using guide catheter (Johnson and Johnson, New Brunswick, NJ) or the SP catheter was delivered over a 0.014″ guidewire (Thunder, Medtronic PLC, Dublin, Ireland). In addition to the main renal artery, the SP catheter was also used to create lesions in the upper or lower branches of the renal artery (with a diameter > 3 mm). Following the ablation procedure, animals were recovered from anesthesia. All procedures were performed by the same interventionist.

2.3. Necropsy

Animals were sacrificed 14 days after treatment and comprehensive necropsies were performed. Both kidneys were immediately harvested for norepinephrine (NE) analysis. The kidney tissue was placed into liquid nitrogen at −20 °C. The kidney tissue was homogenized, and a single sample of the homogenate was analyzed using an HPLC instrument coupled with an electrochemical detector. The NE concentrations for each animal's left and right kidneys were averaged and reported separately. The serosal surfaces of the intestines were evaluated for evidence of adhesions or study-related changes. The renal arteries were excised and perfusion flushed with Lactated Ringer's solution. Renal arteries from four animals were then stained with 1% Triphenyltetrazolium chloride (TTC) at 35–40 °C for 30 min and lesions were subsequently measured. Renal arteries from the remaining animals were perfusion fixed and then immersed in 10% neutral buffered formalin.

2.4. Histopathology

Renal arteries from 4 representative animals were evaluated using histopathology. Arteries were evaluated for lesion effect, total number of nerves and percentage of affected nerves per quadrant and lesion depth and area with the histopathologist blinded to the treatment protocol. These evaluations were conducted throughout the renal artery (proximal, middle, distal, and into the bifurcations). Each treated renal artery was serially sectioned into 8 to 14 segments at approximately 4 mm intervals and submitted in separate cassettes for paraffin processing. Tissue was processed through a graded series of alcohols and xylenes, and after dehydration, vessels were placed on the slide maintaining orientation. Histologic sections were cut on a rotary microtome at 5 μm thickness mounted on charged slides, and stained with hematoxylin and eosin and modified Movat Pentachrome. Immunohistochemical staining was performed for the recognition of axons within nerve fascicles (neurofilament protein: NFP) and the presence of tyrosine hydroxylase (TH), and was assessed based on an ordinal score from 0 to 2 (0 = no reaction, 1 = weak to moderate reaction, 2 = strong reaction). Treated arteries were sectioned serially every 500 μm. Immunohistologic slides (NFP and TH) were digitized using the Axio Scan.Z1 slide scanner equipped with Zen 2012 software (blue edition) (Zeiss, GmbH), while offline measurements were performed using HALO software (Indica Labs, Corrales, NM). Paraffin sections were assessed for injury to the main renal artery and vein and associated branch vessels, nerves, connective tissue, and adjacent lymph nodes utilizing the semi-quantitative scoring criteria. Sections from the kidney and ureter were submitted in separate cassettes for paraffin processing. In addition, any kidney lesions noted were submitted separately. Ablation sites were selected from each renal artery. Ablation area and distance from lumen to deepest tissue damage were measured using morphometric software (Zen desk 2012 [Zeiss]).

2.5. Statistical analysis

SigmaPlot (version 13, Systat Software, Inc., San Jose, CA, USA) was used for all statistical analyses. Comparisons amongst continuous data included *t*-tests, One Way ANOVA as appropriate. *t*-tests were used to test for significance between control and test kidney [NE] within each treatment group. A One-Way ANOVA was used to test for significant differences in the test kidney [NE] values across treatment groups. Further, a *t*-test was used to test for significant difference in electrode temperatures and percent impedance drop between the EN and SP systems. All text and graphical results are reported as mean ± standard deviation.

3. Results

Sixteen domestic swine were treated per study plan, Supplement Table 1. A total of 162 lesions (EN $n = 80$, SP $n = 82$) were created. No animals in this study were noted to have accessory arteries. In 4 of the animals (SP group) the interventionalist had to vary the actual number of treatments delivered in the branch and/or main due to anatomy (e.g. short landing zone, small vessel diameter) to avoid overlapping treatment. Table 1 shows the animal weights and actual treatment matrix.

3.1. Kidney tissue norepinephrine concentration

Each arm showed statistically significant changes in kidney NE (ng/g) between treated kidneys vs. untreated contralateral control (C): EN8 125.0 ± 159.0 vs. EN8-C 461.2 ± 84.1 (−74 ± 34%, $p = 0.01$), EN12 22.9 ± 14.7 vs. EN12-C 492.8 ± 62.9 (−95 ± 3%, $p < 0.001$), SP8 106.1 ± 78.4 vs. SP8-C 438.5 ± 66.4 (−76 ± 16%, $p < 0.001$), and SP12 72.5 ± 79.8 vs. SP12-C 411.1 ± 96.7 (−82 ± 17%, $p = 0.002$). Individual data is shown in Table 2. Fig. 1 shows there were no statistically significant differences in tissue NE % reductions across each arm based on catheter, anatomic location, and number of lesions ($p = 0.496$).

3.2. Generator and catheter analysis

The average electrode temperature (°C) for the two systems was EN 68.0 ± 0.16, $N = 80$ vs. SP 65.4 ± 4.50, $N = 82$ ($p < 0.001$). The percentage of impedance drop from baseline was −17.3 ± 3.0%, $N = 80$ for EN vs. −13.8 ± 3.2%, $N = 82$ for SP ($p < 0.001$), Supplemental Fig. 1. The EN and SP catheters were visually inspected post-treatment in all cases. There were no electrical or mechanical failures noted with any of the catheters used in this study. Both generators functioned as expected throughout the study. There were no issues encountered with the EN generator, with the exception of one error code (Generator software error) during a RF ablation treatment. A total of 80 lesions were created by activating the EN system 20 times. In all cases, all electrodes were actively maximizing efficiency. Prior to energy delivery the diagnostic mode was used resulting in an average diagnostic temperature rise for all electrodes in this study ($N = 80$) by 4 °C (distal electrodes averaged 3.8 °C, proximal electrodes 4.2 °C). A 2-degree diagnostic temp rise was achieved in all lesion sets confirming good tissue contact. A total of 82 lesions were created using the SP generator by activating the system 29 times. In 15 of these 29 attempts, one or more electrodes were deactivated due to high temperature or anatomical reasons. In two cases, the generator shut down at 13 s, and at 24 s due to high temperature. In both cases, the catheter was repositioned and these lesions were not included in the total lesion count.