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TRANSLATIONAL PERSPECTIVES

A Perspective on the Delivery of Renal Denervation Therapy Based on Pre-Clinical Data

Armando Tellez, MD,[a,b,c] Krista N. Dillon,[a] Brad Hubbard, DVM,[d] Dane A. Brady, MS,[a] Dhanansayan Shanmuganayagam, PhD,[b] Serge D. Rousselle, DVM[a]

CURRENT STATUS OF THE RENAL DENERVATION FIELD

The release of unexpected results from the SYMPLICITY HTN-3 (Renal Denervation in Patients With Uncontrolled Hypertension) trial (1,2) has stimulated continued lively discussions and many hypotheses among believers and skeptics of renal denervation (RDN) therapy as to the potential confounding factors leading to the unanticipated outcome. Post hoc evaluation of the data has furthered these discussions and provided important insights. Hypotheses range from selection criteria of hypertensive enrollees as "resistant to medication" (3) and the Hawthorne effect (4), to catheter design (5), operator inexperience, insufficient amount of therapy delivery (6), and/or imprecise location of delivery, among others. Regardless, the impact of the SYMPLICITY HTN-3 trial was notorious and immediate. A large proportion of already skeptical clinicians were sufficiently swayed, and referrals for the therapy vanished, research suddenly dwindled, scientists divided among believers and nonbelievers along the RDN hypertension therapy fault line, and several companies abruptly terminated their RDN product line. Most other companies involved in the RDN field turned their focus on other products. Meanwhile, believers in RDN (i.e., clinicians, researchers, scientist, engineers, investors, and companies), and most importantly, hopeful patient advocates, anxiously await as Boston Scientific (REDUCE-HTN:REINFORCE [Renal Denervation Using the Vessix Renal Denervation System for the Treatment of Hypertension Study]; NCT02439749) and Medtronic (SPYRAL HTN Global Clinical Trial Program: SPYRAL HTN-OFF MED [Global Clinical Study of Renal Denervation With the Symplicity Spyral Multi-electrode Renal Denervation System in Patients With Uncontrolled Hypertension in the Absence of Antihypertensive Medications]; NCT02439749 and SPYRAL HTN-ON MED [Global Clinical Study of Renal Denervation With the Symplicity Spyral Multi-electrode Renal Denervation System in Patients With Uncontrolled Hypertension on Standard Medical Therapy]; NCT02439775) continue with the next phase of clinical trials.

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RDN EVALUATION IN THE PRE-CLINICAL SETTING

Although multiple models of hypertension are available to the research community (7,8), the normotensive domestic swine has been the most utilized model for the evaluation of RDN technologies (9). Although clinical efficacy markers such as blood pressure reduction and inhibition of the resistance to antihypertensive medication cannot be evaluated in this model, the swine remains the preferred model to evaluate acute and long-term effects of RDN due to the similarities of swine vasculature to human. Furthermore, study of the swine model has

From [a]Alizée Pathology, Thurmont, Maryland; [b]Biomedical & Genomic Research (BGR) Group University of Wisconsin, Madison, Wisconsin; [c]Cátedra de Cardiología y Medicina Vascular, Escuela de Medicina, Tecnológico de Monterrey, Monterrey, Mexico; and the [d]Gateway Medical Innovation Center, Shanghai, China. All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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contributed important information on the anatomy of the periarterial sympathetic renal innervation. For instance, awareness is emerging that renal nerves reside closer to the arterial supply in the distal region of the artery than in the proximal segment of the artery, where their position is also more unpredictable, with possibly important implications for more distal RF delivery.

RENAL DENERVATION SAFETY EVALUATION. Extensive published reports support that the therapy may be safely delivered through the renal artery without long-term adverse effects (10,11). Initial thermal injury to the arterial media (hyalinization) is followed by uneventful healing through benign fibrosis of the wall with very minimal non-stenosing neointima formation and rapid re-endothelialization (**Figure 1**). Although rare occurrences of procedural complications have been noted (i.e., dissections caused by guidewires during catheterization), there are virtually no arterial adverse events reported in published reports in the animal model related to the delivery of radiofrequency (RF) ablation (no thrombosis, dissections, or aneurysm formations attributable to RF energy delivery). Collateral damage to adjacent anatomical structures (i.e., psoas muscle, ureter, intestine) have been observed in the swine model yet have not been correlated to any clinical observation. It is important to note that adjustments to wattage, delivery time, and type of electrodes have largely eliminated these findings in pre-clinical studies. In essence, the swine pre-clinical model not only demonstrated the safety of RF RDN, but also shepherded this therapy to current safer clinical settings.

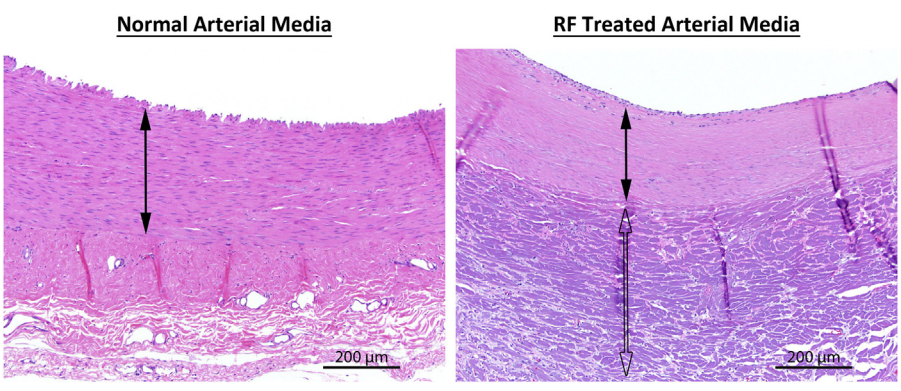
RDN EFFICACY EVALUATION. Delivery of RF energy with single (10) or multiple (12) electrode devices in a spiral fashion along the length of the artery aims for complete ablation of all renal nerves. Pre-clinical surrogates of treatment effectiveness in the pre-clinical model have included evidence of reduction of renal norepinephrine (NE) and histological evidence of distal nerve atrophy.

Although the acquisition and preparation of renal samples for biochemistry vary among pre-clinical studies (i.e., punch biopsy in the cranial, mid and caudal portion of the anterior and posterior part of the kidney, shaving of the anterior cortex, homogenizing the entire kidney), it is understood to be a time-sensitive evaluation. Therefore, minimizing the time between euthanasia and sample collection for snap freezing is crucial to maintaining accuracy and consistency of the data. However, it has been routinely demonstrated in intrastudy comparison that the amount of NE of RF ablation-exposed animals is statistically significantly lower than naive controls (**Figure 2**). This NE level difference has been established to be maintained over time. This effect should be taken cautiously because it needs to be considered that: 1) these models are normotensive and potentially lacking the hyperexcitatory sympathetic activity hypothesized as the rationale behind resistant hypertensive patients (13); 2) there is variability in sample collection; and 3) and renal cortex

ABBREVIATIONS AND ACRONYMS

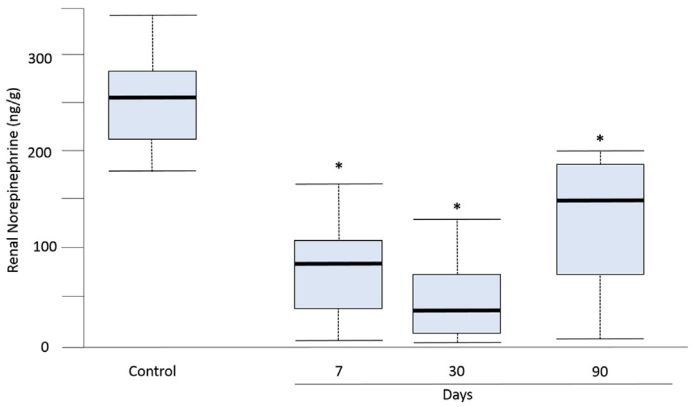
NE = norepinephrine
RDN = renal denervation
RF = radiofrequency

FIGURE 1 Normal and RF-Treated Renal Artery Wall and Media



Representative images of a normal renal artery wall and media **(left image)** displaying a normal arterial thickness **(double-headed arrow)**. Displaying the similar architecture the arterial wall and media of a renal artery following radiofrequency (RF) treatment cycles with a hyalinized media **(right image; solid double-headed arrow)** and necrosis of the surrounding connective tissue (amphophilic to basophilic staining of denatured collagen; **clear double-headed arrow**).

FIGURE 2 Renal Parenchyma Norepinephrine Levels in Naive Control Subjects and After RF Treatment



Representative renal parenchyma norepinephrine levels in naive controls compared with samples following RF treatment at 7, 30, and 90 days in a normotensive porcine model. There is a statistically significant difference between all 3 time points **(*)** compared with control.

NE has not been studied as a translatable counterpart to the serum equivalent in human studies; there has not been a study that demonstrates what is an established baseline of NE in the porcine model or one that compares baseline pre-clinical parameters between all pre-clinical conditions of sampling, breed, age, weight among other parameters that could impact NE baseline levels.

Histological analysis of the nerve response both acutely and at chronic follow-up post-RF treatment reveals that ablated (necrotic) nerves become remodeled through a progressive fibrotic response within and around the perineurium with evidence of sclerotic and likely abortive nerve "sprouting" proximal to and at the ablation site. This response has been termed "neuromatous regeneration" and is analogous to neuromas observed post-nerve amputations. This disorganized "regenerative" response begins quite early in the process with evidence of nerve tangles extending within the reactive perineurium as early as 7 days following RF ablation. This phenomenon is believed to be an attempt of the injured nerve to restore nerve continuity and bypass the ablated area. However, the chronic morphological outcome is consistent with that of a mostly nonfunctional (14) neuromatous response. However, further research needs to be conducted to assess the actual functional importance of these nerve tangles and their long-term significance. The evidence of distal atrophy is to-date the most compelling indication of effective nerve ablation (**Figure 3**). Nevertheless, the potential for functional regeneration exists and was pointed to in the first RDN publication in *Lancet* in 2009 (15) with the statement: "As shown in renal transplantation, renal sympathetic efferent nerves might be able to regrow after injury, indicating that the procedure could have finite time limits in its physiological effects" and restated in the SYMPLICITY HTN-2 trial: "One problem is that sympathetic nerve regrowth might mitigate the treatment effect" (16). This potential should be further investigated.

NEW AREAS OF EXPLORATION

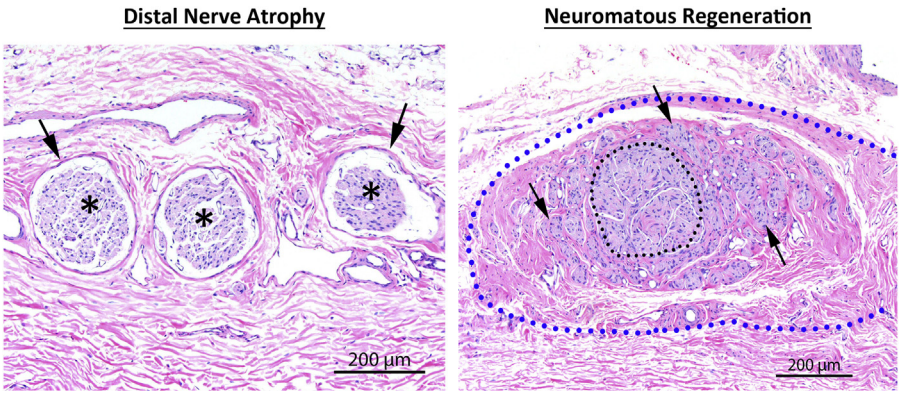
The release of the SYMPLICITY HTN-3 trial's "inconclusive" conclusions resulted in a dramatic decrease in pre-clinical RDN research. However, this technology holds untapped and yet-unresearched potentials. The latest generation of devices allows multiple simultaneous treatment delivery points and/or aims for more distal delivery based on evidence that renal nerves are closer to the arterial wall in the distal part of the artery. Advancements in RDN catheter engineering has lured the developers of these technologies to explore new areas of potential RF delivery (i.e., diabetes, arrhythmias, heart failure, and so on [17-20]). Such territories include the pulmonary arteries (pulmonary hypertension), carotid arteries (hypertension), as well as arteries in the gastrointestinal system (e.g., gastric, pancreatic arteries). Although the clinical endpoint is different for each anatomic location, the physiological lever is still the interruption of the electrical signal between the end organ and the central nervous system. Investigators are also exploring new delivery modalities (i.e., Ureter access, adventitial direct delivery of RF energy) and alternate agents for denervation (i.e., ultrasound, cryoablation, radiotherapy, chemical ablation).

A clear practical limitation of current denervation techniques is the lack of any immediate functional or visual feedback informing the operator that the therapy was appropriately delivered. This limitation has prompted some researchers to focus on technologies for accurate in vivo mapping of targeted nerves and monitoring/probing of their functional response and impairment upon RDN therapy.

OBJECTIVE IN CURRENT NEW CLINICAL TRIAL DESIGN

Taking a lesson from past trials, 2 major players in the field of RDN, Boston Scientific and Medtronic, have refocused objectives, and are now enrolling. Boston Scientific is currently working to enroll 100 patients

FIGURE 3 Nerve Atrophy and Neuromatous Regeneration



To the **left**, representative histological images of nerve atrophy. Nerves distal to the location of delivery of the radiofrequency therapy typically show histological evidence of atrophy **(asterisks and solid arrows)**. To the **right**, representative image of neuromatous regeneration easily identifiable with conventional stains (hematoxylin and eosin [H&E]) as increased fibrous tissue response **(blue dotted line)** and nerve "sprouts" **(solid arrows)** disrupting what was the original anatomy of the nerve **(black dotted line)**.

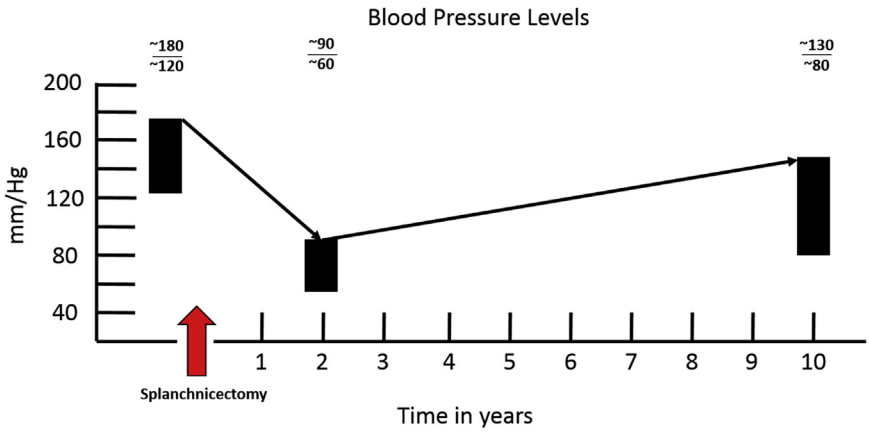
in the REDUCE-HTN:REINFORCE trial, which is a randomized, sham-controlled, multicenter study designed to isolate and demonstrate the effects of the Vessix renal denervation system (Boston Scientific, Natick, Massachusetts). In this trial, patients will undergo a 4-week washout period before enrollment in which they will stop taking all hypertension medications. The primary efficacy assessment will be the mean reduction of average 24-h ambulatory systolic blood pressure 8 weeks post-randomization. This design is intended to isolate the effect of RDN therapy removed from the confounding effects of continued antihypertensive medication.

Concurrently, Medtronic has designed the next step in the evaluation of this therapy. The SPYRAL HTN Global Clinical Trial Program consists of a phase approach to determine the effects of renal denervation in patients or those whose hypertension has been difficult to manage. The first phase will consist of 2 parallel studies, the SPYRAL HTN-OFF MED (NCT02439749) and SPYRAL HTN-ON MED (NCT02439775) trials. These studies are of a randomized (1:1 renal denervation/sham-control group), multicenter design aiming to enroll 120 and 100 patients, respectively (21–23). The SPYRAL HTN-OFF MED study intends to demonstrate the real effect of renal denervation in hypertensive patients without additional hypertension treatment. The study design will use a washout period of 4 weeks before randomization, following the patients up to 12 months. The SPYRAL HTN-ON MED study is designed to evaluate the application of renal denervation in a clinical practice setting in which the pharmacological management of patients is required. In this case, the enrolled patients will be subject to 3 standardized medications. Both studies will be performed using the third-generation Symplicity Spyral renal denervation catheter (Medtronic, Dublin, Ireland). The results of these 2 studies will determine the design of the next phase in the SPYRAL HTN Global Clinical Trial Program. By design, these studies intend to avoid points that were considered potential culprits of the results of the SYMPLICITY HTN-3 trial (i.e., they will avoid requirement of maximum drug dose, measure drug prescription adherence, and produce a theoretical decrease in comorbidities in patients due to less severe hypertensive patient enrollment) (24).

COULD THE FIELD OF RDN BENEFIT FROM A NEW PERSPECTIVE, A NEW PROTOCOL?

The current paradigm for RF RDN is based on a single RDN delivery. As far back as TCT 2010, Mazor et al. (25) presented histological data demonstrating the progressive decrease in larger nerves count and increase in smaller nerves (interpreted as possible evidence of atrophy and/or regeneration) post-denervation with the Vessix balloon in a swine model at long-term follow-up. This was an early indication that nerve microanatomy is measurably altered by RDN treatment. Furthermore, in the first reports of surgical sympathectomy, a pioneering approach for the ablation of renal nerves, Smithwick

FIGURE 4 Blood Pressure Data Over a Period of 10 Years

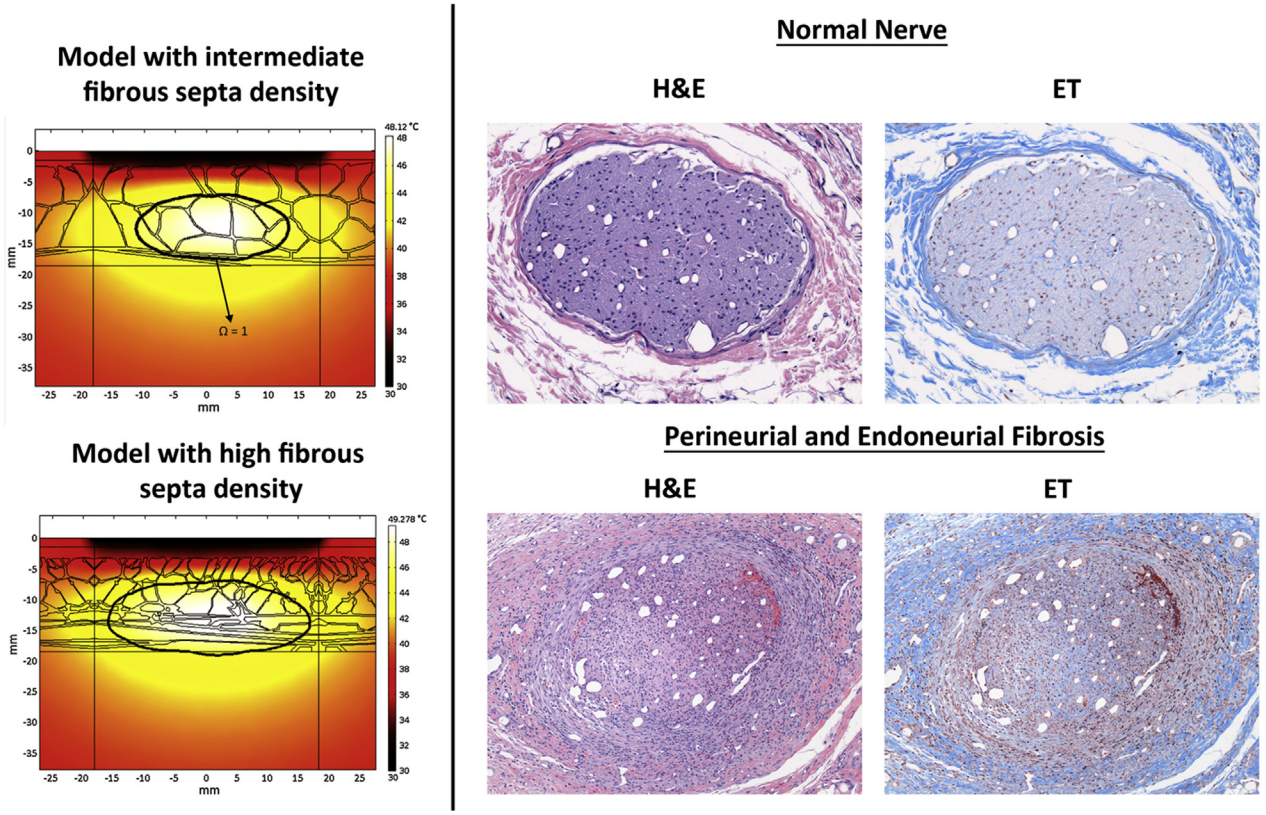


Blood pressure data over a period of 10 years after splanchnicectomy presented by Dr. Reginald H. Smithwick in 1953. This schematic presents the significant blood pressure reduction following the surgical procedure with the lowest level at 2-year follow-up. Ten years later, a steady progressive increase in blood pressure was observed. Although it never reached back to the initial blood pressure, these data suggest that a compensatory process occurred over time despite the surgically removed renal nerves. Modified and adapted with permission from Smithwick and Thompson (26).

and Thompson (26) presented the long-term (10 years) follow-up blood pressure of one patient in which the surgeon physically removed all the nerves surrounding the renal artery. Subsequently, there was a decrease in systolic blood pressure to 88 mm Hg 2 years post-surgery and a rebound to 132 mm Hg after 10 years (**Figure 4**) (26). Such findings raise the question of the feasibility of permanent "denervation" via a catheter-based approach considering that surgical ablation can be followed by a late rebound in blood pressure. Clearly the reasons for such a long-term outcome could be multiple, such as nerve regeneration, local renorenal adaptation involving distal ganglia and alternate sympathetic pathways, non-renal response, etc. Nerve regeneration has been demonstrated post-RF RDN therapy, and whereas microscopic morphology suggests that the neuromatous tangles that appear in the animal model would not yield complete functional recovery, it is possible that some recovery does occur over time. Perhaps the technology, enrollment, and the operators are not the only factors for mitigated results. It remains possible that we are delivering the therapy in an optimal manner but are dismissing what the pre-clinical data have been telling us since the initial studies, namely, through the more recent understanding of the histological response in the animal model, based on this understanding, should a new therapeutic paradigm be considered?

Since the initial evaluations of the effects of RF delivery to the renal arteries in animal models, it has been consistently demonstrated that the healing response primarily entails an increase in fibrous tissue around and within the injured nerve. This response begins early after treatment and is very pronounced at the sites of direct and overwhelming thermal injury. The resulting enlargement of the perineurium is accompanied by regenerative sprouting, resulting in a complete alteration of the normal anatomical structure. This progressive neuromatous response is consistently observed in the pre-clinical setting regardless of the technology employed or time point evaluated (at earlier time periods, special stains are required). The nerve architecture following nerve denervation is completely different than in the naive nerve. The robust fibrous response entraps regenerative nerve bundles in the perineurium and separates any residual nerve fibers within the nerve (endoneurium). Fibrous tissue characteristically lacks the amount of hydration present in viable normal tissue, resulting in a tissue that is potentially more prone to subsequent thermal damage. This was described by Gonzalez-Suarez et al. (27) in a computational modeling study in which fibrous tissue increased the intensity and extent of the energy delivered by favoring flux of electric current and increasing the intensity of the electric field. Furthermore, Liu et al. (28) characterized the "oven effect" that fibrous tissue produces as a distinguishing

FIGURE 5 Fibrous Septa Temperature Distribution and Fibrosis in a Normal and RF-Treated Nerve



On the **left side** of the image, we see the representative image of temperature distribution at steady state (t = 8,000 s) for an intermediate **(above)** and high **(below)** density fibrous septa architecture in a 2-dimensional model of subcutaneous tissue and muscle subjected to a relatively long, constant, low-power radiofrequency (RF) treatment. **Solid line contours** denote the extent of irreversible thermal damage (modified and adapted with permission from Gonzalez-Suarez et al. [27]). On the **right side** of the image, we can observe a representative image of a normal nerve stained with H&E **(left)** and elastin trichrome (ET) **(right)**. By contrast, **below** is a representative image of a nerve subjected to RF ablation with an evident perineurial and endoneurial fibrosis. The experiment by Gonzalez-Suarez et al. (27) suggests that the increase of fibrous tissue within and around the nerve may be an adjuvant in a more efficacious delivery of RF energy in a subsequent therapy session.

feature in delivering RF energy. We, as well as other laboratories, have demonstrated that immediately following RF treatment, tissue collagen is severely denatured (hyalinization), which is followed by remodeling and gradual fibrosis within 30 to 45 days after the RF ablation (**Figure 5**) (14,29,30).

From these observations follows the hypothesis that delivering a single course of RF RDN may not be sufficient to reach permanent long-term ablation. We hypothesize that a judiciously timed second hit may do better than consolidate an initial treatment and would leverage the fibrous tissue response to maximize ablation in a territory rendered more susceptible to thermal injury. Considering that under current technology, there is no immediate feedback to the operator on the effectiveness of denervation therapy delivery, and that there is pre-clinical evidence of an attempt at nerve regrowth, it would seems premature to label the therapy as "ineffective" or a patient a "nonresponder" until the extent of RF therapy may fully be ascertained and deemed inadequate or until a second course demonstrates that no substantial gain is derived. Terumo and its Iberis catheter (Terumo, Tokyo, Japan) have presented "double ablation therapy" data in a pre-clinical model; however, this design evaluated the safety of twice the ablation time (120 s vs. 240 s) in a continuous fashion without an intervening healing period rather than 2 separate treatments. The option of delivering RDN ablation in a "2-step fashion" has not been investigated to this day.

A single study indirectly alluded to this "one-two punch theory" in a publication from Prochnau et al. (31) in which they presented the data of 10 patients

treated with RF therapy and labelled as "non-responders" to RF RDN and subsequently treated with RDN cryoablation. These patients later demonstrated a decrease in office blood pressure at 3, 6, and 12 months (31). Our intent is not to suggest that one technology is better than another (cryoablation vs. radiofrequency), but rather to press forward the concept that performing RDN by cryoablation in nerves that were not naive may greatly potentiate thermal injury and denervation effectiveness. The cryoablation was delivered in already damaged and fibrotic nerves that we hypothesize would be more susceptible to denervation therapies due to an increase of fibrous tissue in the surrounding perineurium and within the nerve itself, the decrease of hydration of the fibrous tissue due to the neuromatous "sprouting," and last but not least, the likely injury to nerves previously spared. This approach has not been evaluated in the clinical or pre-clinical setting. The experimental models have demonstrated that the first RDN may be setting the stage for a more powerful and more sweeping second ablation hit.

CONCLUSIONS

The field of RDN was nearly halted upon release of the SYMPLICITY HTN-3 clinical trial results. Nonetheless, the scientific community continues to explore new device designs, therapeutic areas, territories, and pathologies. The impending results from on-going pivotal clinical trials will produce a new panorama and clearer insights on the unmitigated effects of the delivery of RF energy in hypertensive patients. From the knowledge that fibrous tissue is more conductive than other tissue elements derives the hypothesis that increased fibrous response around healing nerves may exacerbate the thermal effect of RF energy. The hypothesis of a potentially more effective and more durable "one-two punch" delivery of RF RDN is a paradigm shift worth investigating.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Armando Tellez, Alizée Pathology, 20 Frederick Road, Thurmont, Maryland 21788. E-mail: atellez@alizeepathology.com.

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