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VIEWPOINT

Methodological Standardization for the Pre-Clinical Evaluation of Renal Sympathetic Denervation

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ABSTRACT

Transcatheter ablation of renal autonomic nerves is a viable option for the treatment of resistant arterial hypertension; however, structured pre-clinical evaluation with standardization of analytical procedures remains a clear gap in this field. Here we discuss the topics relevant to the pre-clinical model for the evaluation of renal denervation (RDN) devices and report methodologies and criteria toward standardization of the safety and efficacy assessment, including histopathological evaluations of the renal artery, periarterial nerves, and associated periadventitial tissues. The pre-clinical swine renal artery model can be used effectively to assess both the safety and efficacy of RDN technologies. Assessment of the efficacy of RDN modalities primarily focuses on the determination of the depth of penetration of treatment-related injury (e.g., necrosis) of the periarterial tissues and its relationship (i.e., location and distance) and the effect on the associated renal nerves and the correlation thereof with proxy biomarkers including renal norepinephrine concentrations and nerve-specific immunohistochemical stains (e.g., tyrosine hydroxylase). The safety evaluation of RDN technologies involves assessing for adverse effects on tissues local to the site of treatment (i.e., on the arterial wall) as well as tissues at a distance (e.g., soft tissue, veins, arterial branches, skeletal muscle, adrenal gland, ureters). Increasing experience will help to create a standardized means of examining all arterial beds subject to ablative energy and in doing so enable us to proceed to optimize the development and assessment of these emerging technologies. (J Am Coll Cardiol Intv 2014;7:1184–93) © 2014 by the American College of Cardiology Foundation.

The renal autonomic nervous system plays a major role in the development of arterial hypertension (1). Despite the adoption of contemporary pharmacological treatment, a substantial proportion of patients remain at high risk of subsequent cardiovascular and cerebrovascular events due to unexplained resistance to drug treatment (2). Renal sympathetic denervation has recently been

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introduced as a promising option for the treatment of resistant hypertension. Indeed, catheter-based radiofrequency renal denervation (RDN) has demonstrated effectiveness in clinical studies (3). The increasing prevalence of patients with resistant hypertension on a global scale (2) and the appeal of definitive intervention without lifelong obligate adherence to repeated drug dosing has generated a fierce demand to refine current catheter-based RDN procedures and technologies. To this effect, a variety of technological innovations such as radiofrequency and ultrasound catheters, catheter-based microinfusion of neurotoxic drugs, and externally applied focused ultrasound have been developed, and pre-clinical studies for those devices are ongoing (4). The main objective of these technological endeavors pertains to the effective destruction of periarterial sympathetic nerves while preserving arterial morphology and renal function.

In this regard, histopathological assessment of the renal vasculature, along with biomarker analysis of hormones and neurotransmitters, surrounding sympathetic nerves and other regional soft-tissue structures is critically important. However, there remains a clear lack of standardization with respect to the histopathological assessment of these tissues after denervation procedures. Most recently, the failure of the first randomized, sham-controlled clinical trial (SYMPLICITY HTN-3 [Renal Denervation in Patients With Uncontrolled Hypertension]) to reach its primary efficacy endpoint at 6 months underscores the need to revisit existing pre-clinical animal models (5) because there is no marker of procedural efficacy (i.e., confirmation of effective and complete denervation) in humans. In this regard, we aim to establish standardized and reproducible methodology and criteria for histopathological evaluation after renal sympathetic denervation.

ANIMAL MODEL SYSTEMS

There are a number of means of applying energy to the arterial bed and a number of animal models in which such energies can be applied. The early literature in this field dates back to the groundbreaking work of Goldblatt et al. (6) who in the 1930s imposed unilateral or bilateral renal arterial constriction to provoke ischemia and the release of renin to induce hypertension. Their work in dogs defined renal vascular hypertension, helped to define the renin-angiotensin-aldosterone system, and was followed soon thereafter by a series of experiments demonstrating surgical sympathectomy as a possible therapeutic intervention. Other renal injury models emerged including complete ablation or excision of a kidney or infusion of nephrotoxins systemically or locally (7). Other species were considered including small rodents, especially the rat, and occasionally the rabbit (8). As percutaneous technologies have emerged, swine has become a preferred target. Although the bulk of studies are performed in intact animals, it will be increasingly the case that animals with modified renal vasculature and preceding hypertension will be considered. As these models emerge, careful comparison to control states must be attained. Such a definition needs to include not only architecture at a defined period of time but the temporal and spatial kinetics of the dynamics and recognition of systemic and circulating effects. These models therefore expand dramatically the tools available to examine ablative technologies but also simultaneously expand the challenges of careful and precise delineation of effects. Disease models necessarily disrupt the normal architecture, and our view of the normal state is inadequate. Each applied neurotoxic effect is accompanied by idiosyncratic changes, and these must be fully defined before we can proceed with understanding how therapeutic interventions play a role. It will now be especially important as well to track effects over time; we do not know, for example, whether there is recovery of neural ablation. Clinical studies seem to foretell sustained effects, but there is not a definitive time when recovery is observed or deemed beyond approach. As disease models are used, these time-dynamic effects will need to be pursued further.

ABBREVIATIONS AND ACRONYMS

CGRP = calcitonin gene-related peptide

H&E = hematoxylin and eosin

RDN = renal denervation

TH = tyrosine hydroxylase

TTC = 2,3,5-triphenyltetrazolium chloride

PORCINE RENOVASCULAR ANATOMY

Although animal models for the assessment of renal sympathetic denervation remain under development, the swine model is the most frequently used because of its similarity to the renovascular anatomy and size in humans (9). Nevertheless, descriptions of porcine perirenal nerve anatomy remain meager. We examined 11 normal renal arteries from 11 pigs to elucidate renovascular anatomy in this species. A total of 6 to 8 sections from each renal artery and surrounding tissues were sectioned at 4- to 5-mm intervals after marking with indelible ink of the ventral (orange), dorsal (black), superior (blue), and inferior (green) regions and subjected to routine tissue processing, paraffin embedding, sectioning at 4 to 5 μm, and staining with hematoxylin and eosin (H&E) and Movat pertachrome stains. Digital images were prepared from H&E-stained sections