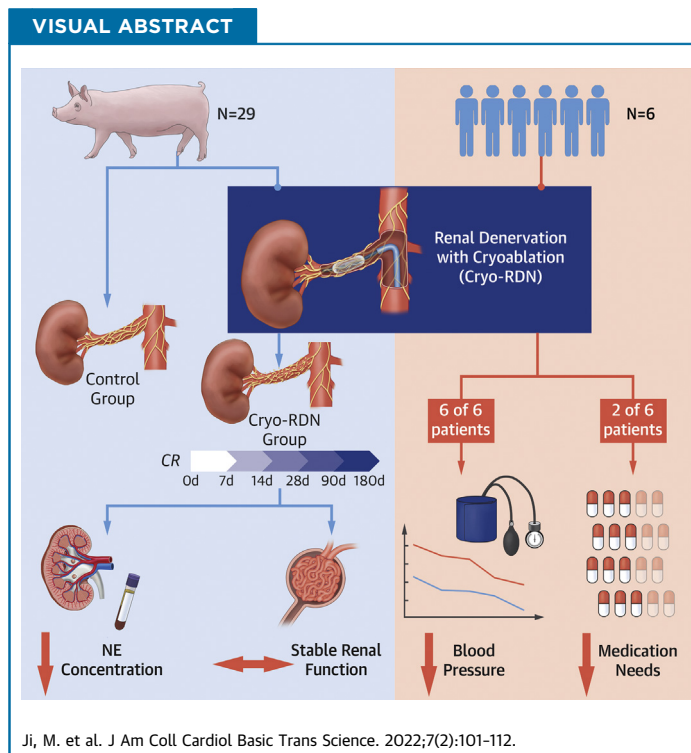**CLINICAL RESEARCH**

Validation of a Novel Renal Denervation System With Cryoablation

A Preclinical Study and Case Series

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VISUAL ABSTRACT

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HIGHLIGHTS

- With the concept of cryoablation, we innovatively applied liquid nitrogen in RDN and designed a dedicated balloon catheter and system for Cryo-RDN.

- In a swine model, this Cryo-RDN system demonstrated device-related safety and efficacy. The extent and depth of nerve ablation were efficient and stable. Sustained decreases in renal and serum norepinephrine also suggested effective ablation in the long term.

- Cryo-RDN also met safety endpoints in 6 patients with resistant hypertension. The 24-hour ambulatory blood pressure and office blood pressure of all 6 patients were reduced after 6 months compared with their baseline values, providing clinical support for further large-scale clinical studies.

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ABBREVIATIONS AND ACRONYMS

24-h ABPM = 24-hour ambulatory blood pressure monitoring

BP = blood pressure

Cryo-RDN = renal denervation with cryoablation

DBP = diastolic blood pressure

eGFR = estimated glomerular filtration rate

Fr = French

NE = norepinephrine

OBP = office blood pressure

RDN = renal denervation

RH = resistant hypertension

SBP = systolic blood pressure

SNS = sympathetic nerve system

TH = tyrosine hydroxylase

SUMMARY

Recently, we designed a renal denervation with cryoablation (Cryo-RDN) system using liquid nitrogen and proved its short-term safety and effectiveness. In this study, we first conducted a 6-month follow-up in a swine model. Renal sympathetic nerve activity remained at a significantly lower level than that of the control group after 6 months. In patients with resistant hypertension, Cryo-RDN demonstrated preliminary safety. Renal function fluctuations and vascular-related complications were not detected. In addition, the average 24-hour systolic and diastolic blood pressure decreased by 12.17 ± 8.35 mm Hg and 8.50 ± 3.83 mm Hg at the 6-month follow-up, respectively, compared with their baseline values. (J Am Coll Cardiol Basic Trans Science 2022;7:101–112) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

More than one billion people worldwide are living with hypertension, and the societal and economic burden of long-term medications and related complications are extremely high[1]. Although drug treatment of hypertension has long been standardized and accepted, approximately 12%-18% of patients are either intolerant of or resistant to medical therapy, and drug adherence is far from satisfactory.[2-4] These observations have brought attention to the importance of device-based antihypertensive therapy, which is a "one-time" treatment with long-term effectiveness.

Among these, renal denervation (RDN), which suppresses the overactivated sympathetic nerve system (SNS), is now the most studied and promising interventional antihypertensive treatment.[5-7] Most of the existing studies are based on radiofrequency and ultrasound ablation and have confirmed the clinical and cost effectiveness of this treatment.[8-11] Therefore, we have gradually begun to explore other candidate energy sources suitable for RDN.

Cryoablation induces apoptosis by causing intracellular dehydration and has been widely used in the treatment of other diseases, such as cancer and arrhythmia. Thus, we originally used liquid nitrogen as a freezing energy source to ablate renal arteries and developed a cryoablation balloon catheter for RDN. Our previous study confirmed the short-term safety and efficacy of a prototype system applied in a swine model.[12] Recently, we optimized the parameters and design of the cryoablation system. In this study, we aimed to verify the safety and efficacy of RDN with cryoablation (Cryo-RDN) in a longer-term swine model and to present the first human experience in patients with resistant hypertension (RH).

METHODS

CRYO-RDN CATHETER. We used a modified Cryo-RDN balloon catheter specific for RDN with available balloon diameters of 4, 5, 6, and 7 mm. The internal structure of the balloon consists of a highly efficient, double-coiled freezing element and a rewarming system (**Figure 1A**). During cryoablation, ultra-low-temperature liquid nitrogen flows inside the coiled freezing element and absorbs heat from the surrounding area. The temperature outside the balloon decreases accordingly (**Figure 1B**). Warm nitrogen gas is used as the rewarming medium during thawing.

GENERAL CRYO-RDN PROCEDURE. For the swine model, all swine were anesthetized using ketamine (0.3 mg/kg) and diazepam (5 mg/kg) to minimize suffering. Volatile anesthetic with isoflurane (1.5%-2%) was used to maintain general anesthesia. For patients, Cryo-RDN was conducted under conscious sedation. Intraprocedural anticoagulation consisting of heparin at 100 U/kg was administered to both swine and patients to prevent thrombosis.

The general operation procedure for both swine and patients was as follows: a 10-F dedicated sheath was inserted into the right femoral artery for the insertion of a 6-F Judkins right (JR4.0) catheter (Cordis, Johnson & Johnson) into the renal arteries. Bilateral renal angiography was first performed to confirm the anatomy and diameter of the main renal artery and the site of cryoablation. The 6-F catheter was replaced with an 8-F dedicated guiding catheter. A Cryo-RDN balloon catheter was placed as distal as possible in the main renal arteries. The balloon was inflated; after confirming that renal blood flow was totally blocked, cryoablation was conducted. Cryo-RDN was performed for 3 minutes in each artery, reducing the

FIGURE 1 Schematic Diagram of the Cryo-RDN System
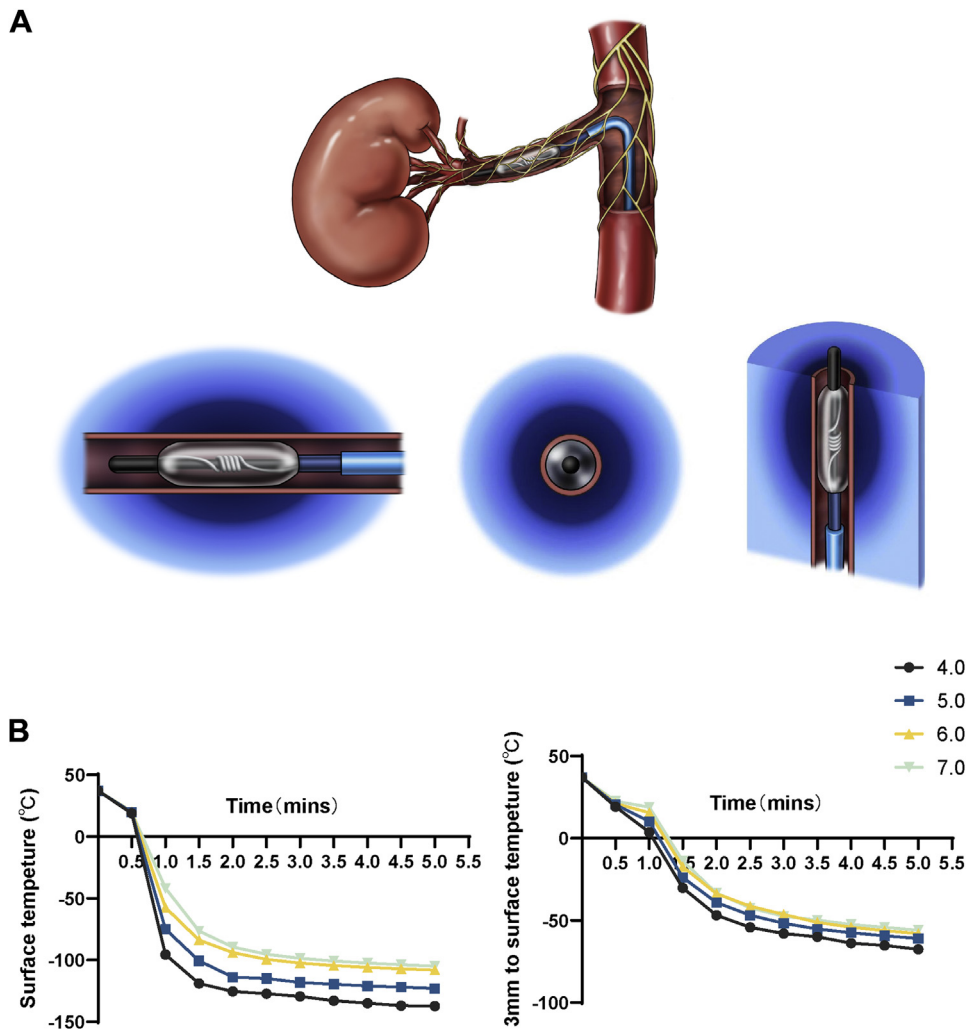
(A) Illustration of the Cryo-RDN catheter in the renal artery (lateral view, anterior view, and sectional axonometric view of the balloon during cryoablation). (B) Temperature change curve of the balloon surface and 3 mm outside the balloon after the start of cryoablation. The balloon consists of a double-coiled high-efficiency freezing and thawing element. During cryoablation, liquid nitrogen at ultralow temperature flows inside the coil freezing element and absorbs heat from surrounding tissues. Cryo-RDN = renal denervation with cryoablation.

temperature to -95°C (ranging from -80°C to -115°C), followed by 1.5 minutes of rewarming. The balloon catheter was deflated and withdrawn, and renal angiography was again performed as a final check for renal artery obstruction, thrombus, perforation, or dissection.

PRECLINICAL STUDY DESIGN. The experimental scheme and the use of swine were approved by the Animal Use and Management Ethics Committee of Fudan University. The experimental design and implementation were in accordance with animal welfare guidelines.

Twenty-nine Shanghai White pigs (mean body weight: 45 ± 5 kg) were randomly assigned to 5 Cryo-RDN groups humanely killed at days 7, 14, 28, 90, and 180 after the operation, designated as the CR-7d (n = 5), CR-14d (n = 5), CR-28d (n = 5), CR-90d (n = 5), and CR-180d (n = 5) groups, respectively, or the control group (n = 4). Animals in the control group underwent renal angiography only; those in the Cryo-RDN groups underwent renal angiography plus bilateral Cryo-RDN. Standard lead II electrocardiography was continuously performed and recorded to determine the heart rate and rhythm.

FIGURE 2   Nerve and Tissue Damage Around the Renal Artery
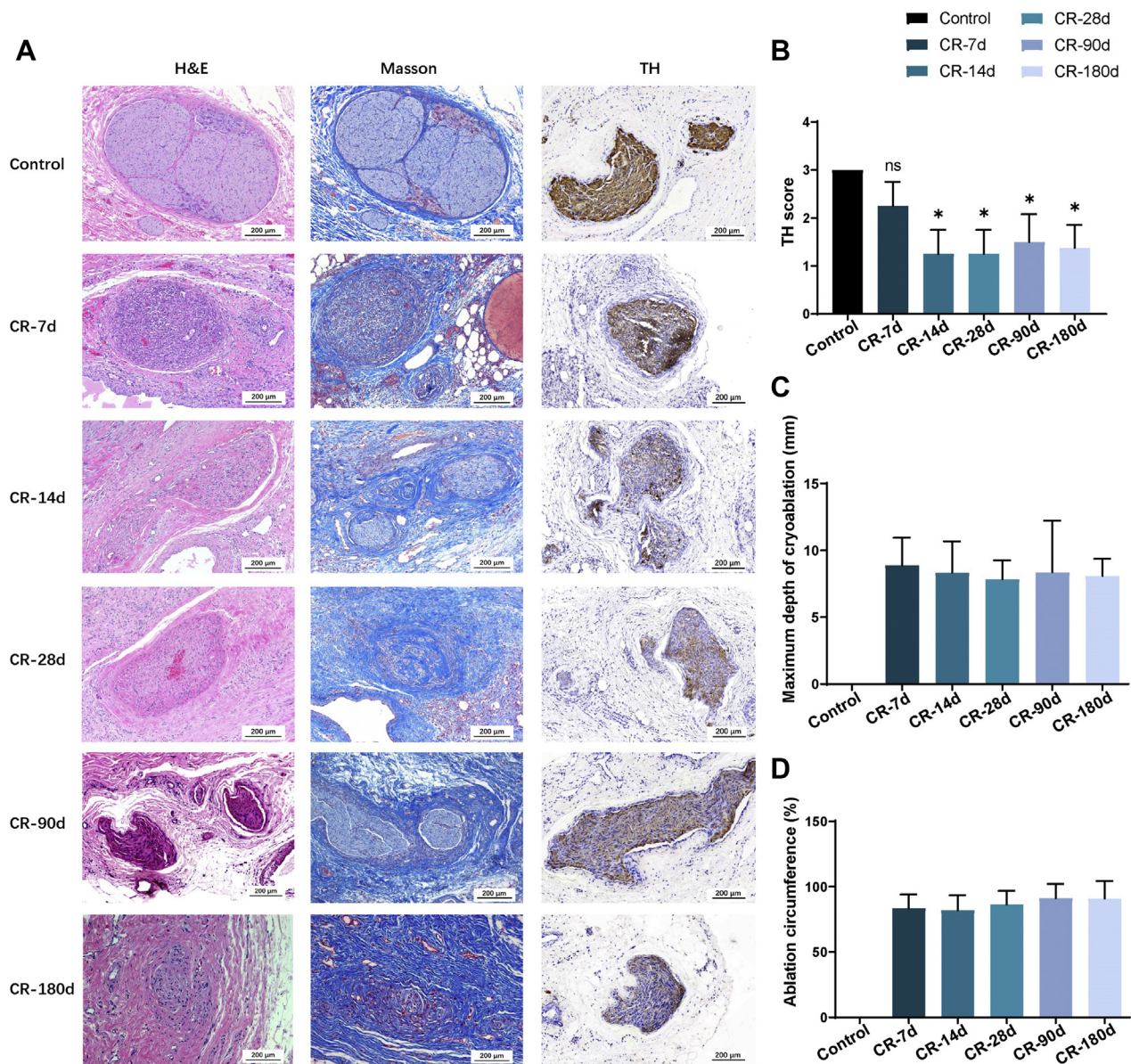
(A) Images of peripheral renal artery injury with hematoxylin & eosin, Masson, and TH staining at different time points. Successful Cryo-RDN was verified by persistent nerve fibrosis and atrophy for up to 180 days. (B) Decrease in TH staining intensity suggesting a decrease in sympathetic activity, scored on a scale from 0 to 3: 0 means no reaction, 1 means patchy/very weak reaction, 2 means weak to moderate reaction, and 3 means strong reaction. The minimum score that represents the most serious functional damage was adopted for each section. (C) Semiquantitative analysis of maximum ablation depth at different times. The maximum ablation depth stabilized at a high level for 180 days. (D) Semiquantitative analysis of maximum ablation circumference at different times. The maximum ablation circumference stabilized at a high level for 180 days. Values are expressed as the mean ± SD by repeated-measures analysis of variance (n = 8 for the control group, n = 10 for the CR-7d group, n = 10 for the CR-14d group, n = 10 for the CR-28d group, n = 10 for the CR-90d group, and n = 10 for the CR-180d group). *P < 0.05. TH = tyrosine hydroxylase; other abbreviation as in **Figure 1**.

HISTOLOGIC ASSESSMENT AND NOREPINEPHRINE DETECTION IN THE SWINE MODEL. At different points, the renal arteries were harvested and evenly divided into 5 parts each. Samples were embedded in paraffin, and slices from each part of the artery were stained with hematoxylin and eosin, Masson, and tyrosine hydroxylase (TH) antibody (ab137869) to assess neural injury via light microscopy. The depth

TABLE 1 Evaluation of Circumferential Injury Induced by Cryo-RDN					
	CR-7d (n = 10)	CR-14d (n = 10)	CR-28d (n = 10)	CR-90d (n = 10)	CR-180d (n = 10)
Injury circumferential extension (%)	83.4 ± 10.7	82.0 ± 11.4	86.4 ± 10.4	91.2 ± 11.0	90.9 ± 13.4
Injured nerve/total nerve (%)	84.0 ± 3.6	94.7 ± 4.7	93.0 ± 6.2	92.0 ± 3.6	89.3 ± 9.3

Values are mean ± SD. Injury circumferential extension (0%-100%) represents 0-360 degrees of injury in 1 cross-sectional image.

Cryo-RDN = renal denervation with cryoablation.

of cryoablation and TH staining intensity were semi-quantitatively measured as previously described to assess the effects of Cryo-RDN.

At the end of the follow-up period, the swine were anesthetized, and 6 pieces of renal cortical tissue and peripheral blood were collected; the norepinephrine (NE) concentration was measured using high-performance liquid chromatography.

FIRST-IN-HUMAN CASE SERIES. This study was reviewed and approved by the ethics committees of Zhongshan Hospital Fudan University and Shanghai Tenth People's Hospital ChiCTR1900020545.

Based on the requirement for the sample size from the Food and Drug Administration, a total of 6 patients (5 males and 1 female) with RH were recruited.[13] Briefly, eligible patients were males or females aged 18-75 years old with a history of consecutive antihypertensive drug intake (≥3 types

of antihypertensive drugs, including diuretics) with no alteration ≥2 weeks (average office systolic blood pressure [SBP] >140 mm Hg with 3 measurements and 24-hour SBP ≥135 mm Hg). Patients also needed to have an estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m² and no history of cardiovascular or cerebrovascular events. Detailed inclusion and exclusion criteria are listed in Supplemental Table 1. Informed consent was obtained before enrollment. Medication intake, 24-hour ambulatory blood pressure monitoring (24-hour ABPM), office blood pressure (OBP), and eGFR at baseline and 1 and 6 months after Cryo-RDN were all recorded and compared. To monitor drug adherence, antihypertensive drugs were distributed to patients every 28 days during follow-up, and any excess medication from the previous distribution was collected at the next distribution. Renal angiography

FIGURE 3 Renal and Serum NE Concentration at Different Times
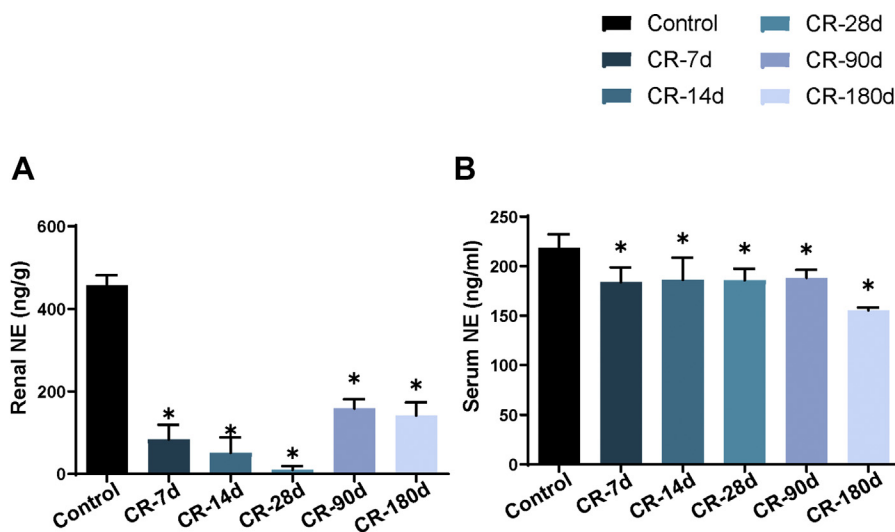
(A) NE concentration in the renal cortex at different times after Cryo-RDN. (B) NE concentration in serum at different times after Cryo-RDN. Values are expressed as the mean ± SD by repeated-measures of analysis of variance (n = 4 for the control group, n = 5 for the CR-7d group, n = 5 for the CR-14d group, n = 5 for the CR-28d group, n = 5 for the CR-90d group, and n = 5 for the CR-180d group). *P < 0.05. NE = norepinephrine; other abbreviations as in Figures 1 and 2.

TABLE 2   Characteristics of Patients at Baseline (N = 6)

Age, y	55.83 ± 9.00
Females	1 (16.7)
Body mass index, kg/m²	26.92 ± 4.94
OBP, mm Hg	
SBP	157.17 ± 15.05
DBP	102.00 ± 9.63
24-h average BP, mm Hg	
SBP	145.83 ± 5.49
DBP	89.67 ± 9.93
Heart beats, beats/min	66.17 ± 7.96
Laboratory results	
eGFR, mL/min/1.73 m²	78.52 ± 12.25
LDL cholesterol, mmol/L	2.30 ± 0.83
Methoxy norepinephrine, pg/mL	87.03 ± 47.58
Aldosterone, pg/mL	156.68 ± 55.27
Comorbidity	
Diabetes mellitus	0 (0)
Coronary artery disease	1 (16.7)
Sleep apnea syndrome	1 (16.7)
Atrial fibrillation	2 (33.3)
Antihypertensive medication therapy	
No. of antihypertensive medications	4.83 ± 0.57
ACEIs/ARBs	6 (100.0)
Calcium channel blockers	5 (83.3)
Diuretics	6 (100.0)
Beta-blockers	6 (100.0)
Centrally acting sympatholytics	1 (16.7)

Values are mean ± SD or n (%). The concentration of methoxy norepinephrine was measured using HPLC.

ACEIs = angiotensin-converting enzyme inhibitors; ARBs = angiotensin receptor blockers; BP = blood pressure; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HPLC = high-performance liquid chromatography; LDL = low-density lipoprotein; OPB = office blood pressure; SBP = systolic blood pressure; other abbreviations as in **Table 1**.

was performed at the 6-month follow-up. The occurrence of device-related adverse events in 6 patients was recorded to evaluate clinical safety.

STATISTICAL ANALYSIS. All data were analyzed using GraphPad Prism 9.0 (GraphPad Software) and are presented as the mean ± SD for continuous variables. Categorical variables at baseline and during the follow-up period are presented as counts and percentages. The data from the animal study were compared with repeated-measures analysis of variance after testing for normal distribution. A value of $P < 0.05$ was considered statistically significant.

RESULTS

PRECLINICAL STUDY. All animals in the cryoablation groups underwent bilateral Cryo-RDN and survived until necropsy without any procedure-related complications. The efficacy of cryoablation was comprehensively evaluated based on histologic and serologic indicators.
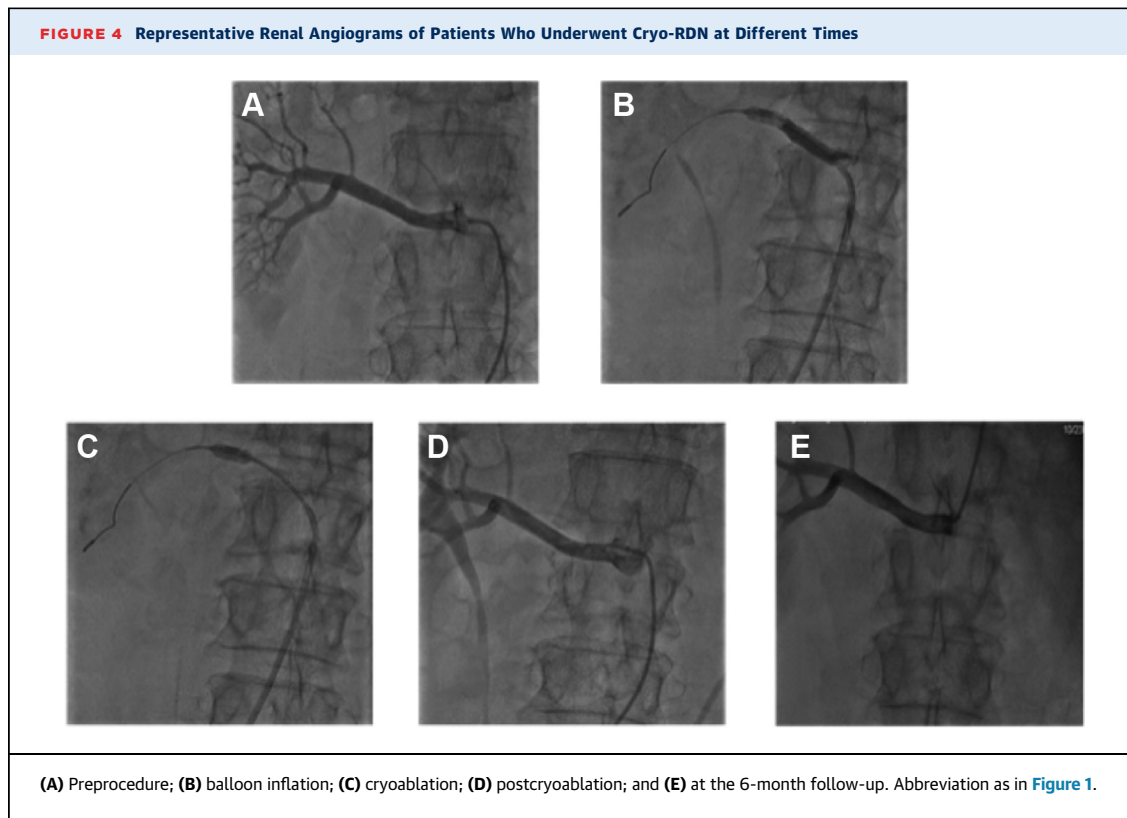
HISTOLOGIC EVALUATION AFTER CRYO-RDN. All 58 renal arteries from the 29 pigs were harvested at different times for histologic analysis. Pathological changes after cryoablation were observed in all vessels in the Cryo-RDN groups (**Figure 2A**). Persistent nerve fibrosis and atrophy lasted up to day 180. As a marker of SNS activity, TH staining intensity decreased after Cryo-RDN and remained at a significantly lower level than that of the control group (**Figure 2B**).

The efficacy of Cryo-RDN with this device was evaluated by analyzing the maximal cryoablation depth between every follow-up, which comparatively ranged from 7.85 ± 1.41 mm to 8.89 ± 2.06 mm ($P \geq$ 0.05; **Figure 2C**). All pigs in the 5 Cryo-RDN groups showed >80% injury in terms of circumferential extension at the median level (**Figure 2D**). Consistent with this finding, the presence of at least 80% nerve damage was observed in all pigs ($P \geq 0.05$) (**Table 1**). Limited muscle damage and lymph node necrosis were reported in 2 pigs but were not safety concerns. No appreciable adverse changes were observed in the renal parenchyma. There was no collateral damage to surrounding structures such as the adrenal gland or intestines.

SNS ACTIVITY ASSESSMENT AFTER CRYO-RDN. Cryo-RDN treatment resulted in a significant suppression of SNS activity in the swine model. Consistent with the decrease of TH staining intensity, on day 7, the renal NE concentration decreased 90% compared with that in the control group and remained significantly lower until day 180. The absolute values and changes in the renal NE concentration are shown in **Figure 3A**. In addition, the serum NE level decreased 10% compared with that in the control group after 7 days and remained stable in all groups after Cryo-RDN (**Figure 3B**).

RESULTS OF THE FIRST-IN-HUMAN CASE SERIES. After obtaining preclinical results in a swine model on device safety and efficacy, we conducted a first-in-human case series of 6 patients with RH. Their baseline and serologic parameters are shown in **Table 2**.

SAFETY ASSESSMENT OF CRYO-RDN. Bilateral Cryo-RDN was successfully performed in all 6 participants and no serious adverse events occurred in the perioperative period. Based on renal angiography, no adverse complications, including endovascular thrombus and vascular injuries, were detected at any point and the vascular diameter of renal arteries was

FIGURE 4 Representative Renal Angiograms of Patients Who Underwent Cryo-RDN at Different Times

(A) Preprocedure; **(B)** balloon inflation; **(C)** cryoablation; **(D)** postcryoablation; and **(E)** at the 6-month follow-up. Abbreviation as in **Figure 1**.

comparable with that in the preoperative period (**Figure 4**).

In addition, adverse nephrotoxic or systemic effects were not reported. The eGFR values remained stable throughout the 6-month follow-up period (baseline: 78.52 ± 12.25 mL/min/1.73 m² vs. 6-month follow-up: 74.50 ± 10.65 mL/min/1.73 m²).
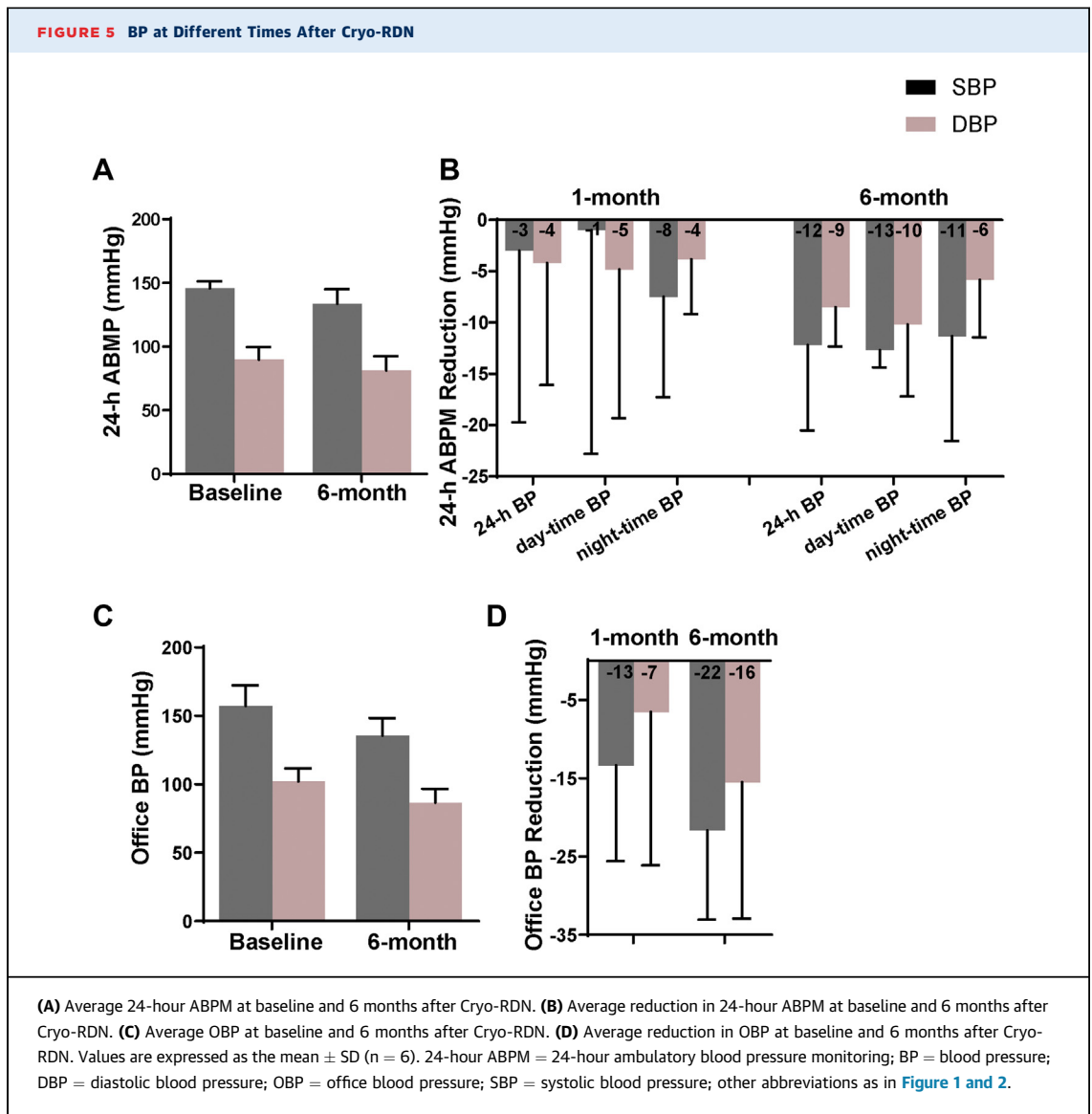
BLOOD PRESSURE PERFORMANCE AFTER CRYO-RDN. At the 1-month follow-up, 24-hour ABPM-related indices decreased slightly compared with baseline values. This tendency was more obvious until 6 months, when the average 24-hour SBP and diastolic blood pressure (DBP) in patients decreased dramatically by 12.17 ± 8.35 mm Hg and 8.50 ± 3.83 mm Hg, respectively (**Figures 5A and 5B, Table 3**). The declining trend in OBP was similar to that in 24-hour ABPM (**Figures 5C and 5D, Table 3**). Individual blood pressure (BP) monitoring data are shown in **Figure 6**.

We counted the antihypertensive drugs returned by our 6 patients and did not find any cases of missed doses or overdosing. Surprisingly, antihypertensive drug reduction was reported in 2 of 6 patients during the follow-up period, where 1 patient was able to reduce 1 type of antihypertensive drug and the other was able to reduce 2 types of antihypertensive drugs (**Table 4**).

DISCUSSION

Based on previous studies, we speculated that cryoablation may also disrupt the over-activated SNS and could be used for RDN. Thus, we designed a new Cryo-RDN system and tested its safety and efficacy. In the long-term follow-up of the swine model, pathological analysis of renal arteries showed that Cryo-RDN effectively damaged sympathetic nerves with no vascular-related damage. Further sustained reduction in NE concentration confirmed the ablation efficiency for at least 6 months. For the following first-in-human case series, Cryo-RDN demonstrated preliminary safety. Patients' average 24-hour SBP and DBP decreased by 12.17 ± 8.35 mm Hg and 8.50 ± 3.83 mm Hg, respectively, over a 6-month follow-up period. Office SBP and DBP decreased by 13.33 ± 12.27 mm Hg and 6.50 ± 19.52 mm Hg after 1 month and 21.67 ± 11.40 mm Hg and 15.50 ± 17.41 mm Hg after 6 months, respectively. No adverse events were reported based on renal function and angiography. This study preliminarily suggested the feasibility of the clinical application of this Cryo-RDN system.

FIGURE 5 BP at Different Times After Cryo-RDN

(A) Average 24-hour ABPM at baseline and 6 months after Cryo-RDN. (B) Average reduction in 24-hour ABPM at baseline and 6 months after Cryo-RDN. (C) Average OBP at baseline and 6 months after Cryo-RDN. (D) Average reduction in OBP at baseline and 6 months after Cryo-RDN. Values are expressed as the mean ± SD (n = 6). 24-hour ABPM = 24-hour ambulatory blood pressure monitoring; BP = blood pressure; DBP = diastolic blood pressure; OBP = office blood pressure; SBP = systolic blood pressure; other abbreviations as in **Figure 1 and 2**.

In this study, we adopted liquid nitrogen as a freezing energy source and proved its effectiveness in a swine model. Based on our pathological analysis, Cryo-RDN with liquid nitrogen successfully induced neuronal apoptosis and nerve fibrosis and maintained an ablation depth of approximately 8 mm for 6 months. Previous autopsy reports have shown that 60%-80% of the peripheral SNS nerves around renal arteries are located within a distribution radius of <6.5 mm, which is within the coverage capacity of Cryo-RDN.[14] This ablation depth is also equivalent to those of radiofrequency and ultrasound, which are 2 of the most studied and accepted ablation energy sources.[15-17]

With this sufficient ablation depth, Cryo-RDN was not associated with device-related adverse events.

Both pathological analysis of the renal artery in swine and angiography of patients with RH showed no vascular complications (thrombosis, dissection, or stenosis). Furthermore, because the entire ablation process for each renal artery was completed in 5 minutes, the risk of kidney ischemia was minimal. Patients' renal function did not fluctuate during follow-up and they reported only mild intraoperative discomfort. For these reasons, the application of liquid nitrogen as a freezing energy source for Cryo-RDN has certain energy potential and implies a safe and effective clinical application in the future.

Additionally, because the ablation coverage percentage is another crucial factor in the efficiency of cryoablation, we designed a balloon catheter.

Pathological results from the swine model showed that the effective ablation circumference reached 80% with a high ablation coverage percentage. As commonly used indicators of sympathetic activity in RDN, TH staining intensity and renal and serum NE concentrations were reduced significantly compared with those in the control group.[18-20] Previous experience in the development of radiofrequency catheters tells us that the ablation efficiency and stability of the antihypertensive effect can be improved by increasing the ablation coverage percentage.[21,22] Thus, in the new-generation, sham-controlled, randomized clinical trials, the SPYRAL HTN-ON MED and HTN-OFF MED studies, a multielectrode radiofrequency catheter was applied, in which RDN revealed its antihypertensive effect with or without medication and long-term persistence.[8,9] This theory has also been supported by study of another balloon RDN catheter, the Paradise Renal Denervation System, which delivers ultrasound energy and was reported to perform better than radiofrequency ablation of the main renal arteries in a randomized, head-to-head trial.[15] Using the same concept, our

Cryo-RDN balloon catheter showed high ablation efficiency.

Consistent with the results from the preclinical animal study, the results from our 6 patients with RH demonstrated the preliminary safety of Cryo-RDN. In
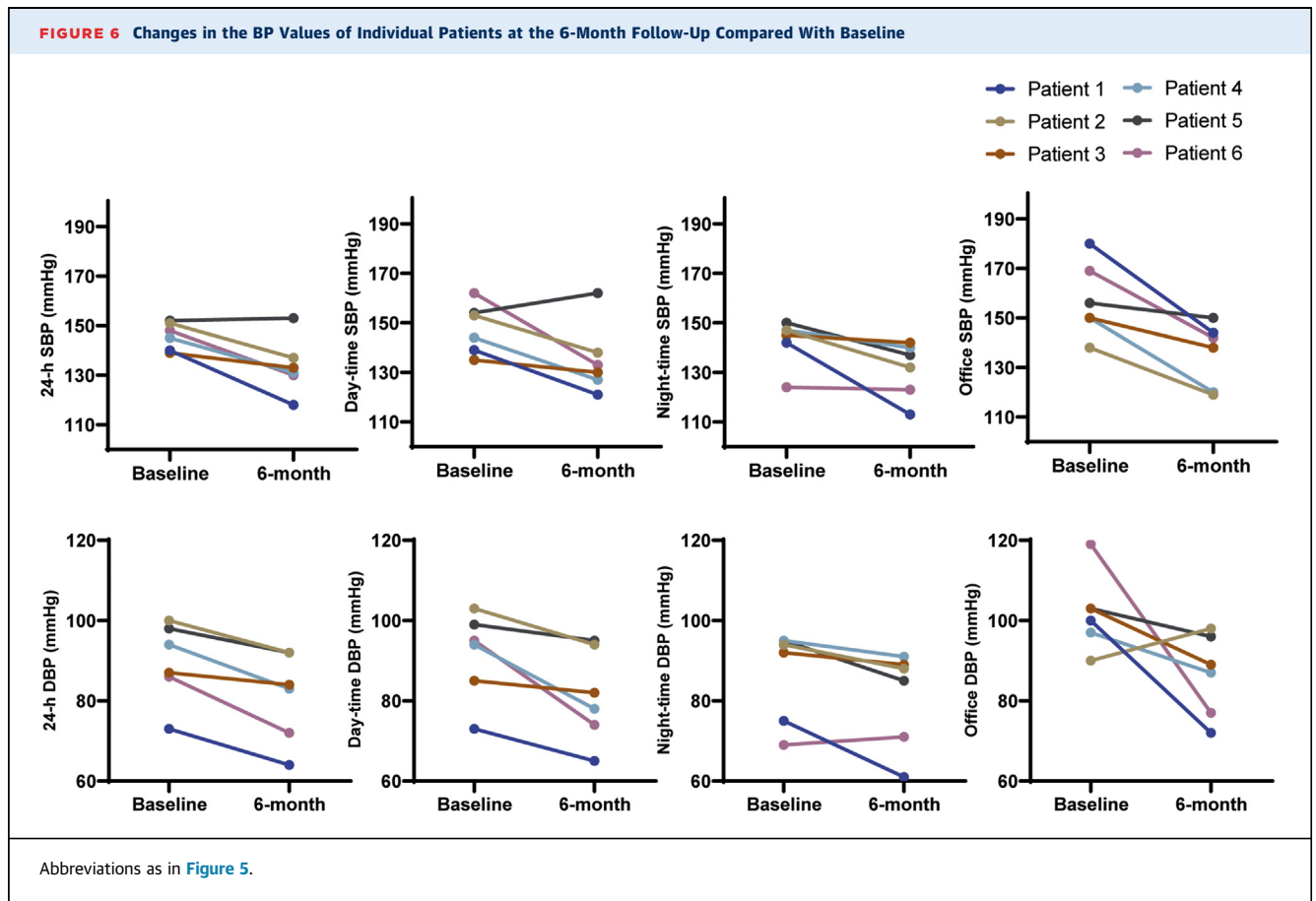
TABLE 3	BP Level, Heart Rate at Baseline and Follow-Ups		
	Baseline	**1 Mo**	**6 Mo**
Ambulatory BP, mm Hg			
24-h SBP	145.83 ± 5.49	142.83 ± 13.44	133.67 ± 11.41
24-h DBP	89.67 ± 9.93	85.50 ± 8.17	81.17 ± 11.18
Daytime SBP	147.83 ± 10.23	146.83 ± 14.82	135.17 ± 14.33
Daytime DBP	91.50 ± 10.88	86.67 ± 6.77	81.33 ± 11.66
Nighttime SBP	142.50 ± 9.44	135.00 ± 11.92	131.17 ± 11.20
Nighttime DBP	86.67 ± 11.57	82.83 ± 10.72	80.83 ± 12.07
Office BP, mm Hg			
Office SBP	157.17 ± 15.05	141.33 ± 18.66	135.50 ± 12.99
Office DBP	102.00 ± 9.63	92.17 ± 11.02	86.50 ± 10.29
Heart rate, beats/min	66.17 ± 7.96	70.50 ± 8.22	72.67 ± 10.19

Values are mean ± SD.
Abbreviations as in Tables 1 and 2.

FIGURE 6 Changes in the BP Values of Individual Patients at the 6-Month Follow-Up Compared With Baseline

Abbreviations as in Figure 5.

TABLE 4   Antihypertensive Agents at Baseline and Follow-Ups

	Baseline	1 Mo	6 Mo
No. of antihypertensive medications	4.83 ± 0.57	3.83 ± 1.60	3.83 ± 1.72
ACEIs/ARBs	6 (100.0)	5 (83.3)	5 (83.3)
Calcium channel blockers	5 (83.3)	5 (83.3)	6 (100.0)
Diuretics	6 (100.0)	4 (66.7)	4 (66.7)
Beta-blockers	6 (100.0)	6 (100.0)	5 (83.3)
Centrally acting sympatholytics	1 (16.7)	1 (16.7)	1 (16.7)

Values are mean ± SD or n (%).

Abbreviations as in **Tables 1 and 2**.

addition, the 24-hour SBP and DBP decreased by 12.17 ± 8.35 mm Hg and 8.50 ± 3.83 mm Hg, respectively, compared with baseline values. Similarly, the office SBP and DBP decreased by 13.33 ± 12.27 mm Hg and 6.50 ± 19.52 mm Hg, respectively, after 1 month and 21.67 ± 11.40 mm Hg and 15.50 ± 17.41 mm Hg, respectively, after 6 months compared with baseline values. These positive trends in BP reduction were similar to the latest SPYRAL and RADIANCE-HTN studies.[8-10,23,24] Because the sample size of patients was relatively small in this preliminary exploration of Cryo-RDN, comparison and interpretation with these studies cannot be made until further confirmation of the results in large randomized controlled trials.

Hypertension as a chronic disease requires long-term medication, and some patients have a certain degree of medication compliance and tolerance problems[25]. These individuals remain at increased risk of target organ damage, morbidity, and mortality despite ongoing antihypertensive drug therapy. This gives rise to device-based interventional antihypertensive treatment. To date, few published articles have focused on cryoablation for RDN. In 2014, Prochnau et al[26] reported a successful antihypertensive effect in 10 patients with RH who failed to show BP reduction after radiofrequency RDN. Although the authors suggested to some extent that cryoablation may be an energy option for RDN, atrial fibrillation cryoablation catheters were used in that study, and only 4 lesions were ablated on each side of the renal artery.

In our experiments, we originally designed a balloon ablation catheter and used liquid nitrogen as a freezing energy source for RDN. This method has several advantages. First, previous reports have indicated that cryoablation is endothelium friendly for arteries.[27-29] Second, based on our results, Cryo-RDN of 1 renal artery took approximately 5 minutes; the overall procedure duration was relatively short. In addition, the operation is simple with a short learning curve, and the procedure is operator friendly.

STUDY LIMITATIONS. Our study has several limitations. First, medication adherence has a dominant influence on BP control, but we did not test for blood concentrations of antihypertensive drugs.[30] Standard antihypertensive drugs were distributed monthly, ensuring medication adherence to a large extent. In addition, a recent study suggested that medication adherence was high in patient follow-up after RDN, which suggests that, to some extent, the BP reduction in our patients after Cryo-RDN was less influenced by drug adherence than by the effect of Cryo-RDN itself.[31] Second, the follow-up of patients with RH was relatively short considering that, thus far, the longest clinical follow-up of RDN in patients with RH was 36 months and demonstrated sustained antihypertensive efficacy, with even further reductions in BP over the follow-up period.[6,32] In addition, as an early feasibility study, the sample size was limited.[13] We have designed a multicenter randomized controlled trial to explore the long-term clinical efficacy of Cryo-RDN that is now ongoing.[33]

CONCLUSIONS

Cryo-RDN with this dedicated cryoablation balloon catheter is safe and effective for sympathetic denervation in a swine model. Preliminary safety was demonstrated in the first-in-human case series, and an antihypertensive effect was reported in 6 patients with RH. Although further long-term randomized clinical trials with larger sample sizes are needed for confirmation, the results of this study indicate that this cryoablation technique with this new device could be a breakthrough in the clinical treatment of RH.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: RDN is performed by suppressing the overactive SNS. The results from recent clinical studies have suggested that RDN is the most promising interventional treatment for RH. Thus far, almost all studies have been based on radiofrequency and ultrasound ablation. Cryoablation induces apoptosis by causing intracellular dehydration and is widely used in the treatment of many other diseases. In this study, we innovatively adopted liquid nitrogen as a freezing energy source to perform Cryo-RDN. The long-term effectiveness of Cryo-RDN was demonstrated in a swine model with stable ablation depth and circumference. In the subsequent first-in-human case series, the safety of Cryo-RDN was preliminarily reported. In addition, 6 patients with RH showed a dramatic BP decrease at the 6-month follow-up, and 2 achieved antihypertensive drug reduction.

TRANSLATIONAL OUTLOOK: More than 1 billion people worldwide are living with hypertension, and approximately 12%-18% of them are either intolerant of or resistant to medical therapy; drug adherence is far from satisfactory. The societal and economic burden of long-term medication and related complications is extremely high. RDN is a "one-time" treatment with long-term effectiveness. Cryoablation has been suggested to have great safety and efficacy in Cryo-RDN; the operation procedure is operator friendly and takes little time. The results of this study indicate that this cryoablation technique with this new device could be a breakthrough in the clinical treatment of RH.

REFERENCES

1. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol.* 2020;16:223–237.

2. Borghi C, Tubach F, De Backer G, et al. Lack of control of hypertension in primary cardiovascular disease prevention in Europe: results from the EURIKA study. *Int J Cardiol.* 2016;218:83–88.

3. Egan BM, Zhao Y, Li J, et al. Prevalence of optimal treatment regimens in patients with apparent treatment-resistant hypertension based on office blood pressure in a community-based practice network. *Hypertension.* 2013;62:691–697.

4. Diaz KM, Booth JN 3rd, Calhoun DA, et al. Healthy lifestyle factors and risk of cardiovascular events and mortality in treatment-resistant hypertension: the Reasons for Geographic and Racial Differences in Stroke study. *Hypertension.* 2014;64:465–471.

5. Krum H, Schlaich M, Whitbourn R, et al. Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. *Lancet.* 2009;373:1275–1281.

6. Esler MD, Bohm M, Sievert H, et al. Catheter-based renal denervation for treatment of patients with treatment-resistant hypertension: 36 month results from the SYMPLICITY HTN-2 randomized clinical trial. *Eur Heart J.* 2014;35:1752–1759.

7. Krum H, Schlaich MP, Sobotka PA, et al. Percutaneous renal denervation in patients with treatment-resistant hypertension: final 3-year report of the Symplicity HTN-1 study. *Lancet.* 2014;383:622–629.

8. Bohm M, Kario K, Kandzari DE, et al. Efficacy of catheter-based renal denervation in the absence of antihypertensive medications (SPYRAL HTN-OFF MED Pivotal): a multicentre, randomised, sham-controlled trial. *Lancet.* 2020;395:1444–1451.

9. Kandzari DE, Bohm M, Mahfoud F, et al. Effect of renal denervation on blood pressure in the presence of antihypertensive drugs: 6-month efficacy and safety results from the SPYRAL HTN-ON MED proof-of-concept randomised trial. *Lancet.* 2018;391:2346–2355.

10. Azizi M, Daemen J, Lobo MD, et al. 12-month results from the unblinded phase of the RADIANCE-HTN SOLO trial of ultrasound renal denervation. *J Am Coll Cardiol Intv.* 2020;13:2922–2933.

11. Azizi M, Schmieder RE, Mahfoud F, et al. Six-month results of treatment-blinded medication titration for hypertension control following randomization to endovascular ultrasound renal denervation or a sham procedure in the RADIANCE-HTN SOLO trial. *Circulation.* 2019; Mar 17;139:2542–2553.

12. Ji M, Shen L, Wu YZ, et al. Short-term safety and efficiency of cryoablation for renal sympathetic denervation in a swine model. *Chin Med J (Engl).* 2015;128:790–794.

13. Investigational Device Exemptions (IDEs) for Early Feasibility Medical Device Clinical Studies, Including Certain First in Human (FIH) Studies. Accessed December 28, 2014. https://www.fda.gov/media/81784/

14. Sakakura K, Ladich E, Cheng Q, et al. Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J Am Coll Cardiol.* 2014;64:635–643.

15. Fengler K, Rommel KP, Blazek S, et al. A three-arm randomized trial of different renal denervation devices and techniques in patients with resistant hypertension (RADIOSOUND-HTN). *Circulation.* 2019;139:590–600.

16. Al Raisi SI, Pouliopoulos J, Barry MT, et al. Evaluation of lesion and thermodynamic characteristics of Symplicity and EnligHTN renal denervation systems in a phantom renal artery model. *EuroIntervention.* 2014;10:277–284.

17. Sakakura K, Roth A, Ladich E, et al. Controlled circumferential renal sympathetic denervation with preservation of the renal arterial wall using intraluminal ultrasound: a next-generation approach for treating sympathetic overactivity. *EuroIntervention.* 2015;10:1230–1238.

18. Cai X, Yang Y, Shen Y, et al. Noninvasive stereotactic radiotherapy for renal denervation in a swine model. *J Am Coll Cardiol.* 2019;74:1697–1709.

19. Wang Q, Guo R, Rong S, et al. Noninvasive renal sympathetic denervation by extracorporeal high-intensity focused ultrasound in a pre-clinical canine model. *J Am Coll Cardiol.* 2013;61:2185–2192.

20. Denfeld QE, Habecker BA, Woodward WR. Measurement of plasma norepinephrine and 3,4-dihydroxyphenylglycol: method development for a translational research study. *BMC Res Notes.* 2018;11:248.

21. Lee CK, Wang TD, Lee YH, et al. Efficacy and Safety of renal denervation for patients with uncontrolled hypertension in Taiwan: 3-year results from the Global SYMPLICITY Registry-Taiwan (GSR-Taiwan). *Acta Cardiol Sin.* 2019;35:618–626.

22. Kandzari DE, Bhatt DL, Brar S, et al. Predictors of blood pressure response in the SYMPLICITY HTN-3 trial. *Eur Heart J.* 2015;36:219–227.

23. Mahfoud F, Bloch MJ, Azizi M, et al. Changes in blood pressure after crossover to

ultrasound renal denervation in patients initially treated with sham in the RADIANCE-HTN SOLO trial. *EuroIntervention*. 2021;17(12): e1024–e1032.

24. Azizi M, Sanghvi K, Saxena M, et al. Ultrasound renal denervation for hypertension resistant to a triple medication pill (RADIANCE-HTN TRIO): a randomised, multicentre, single-blind, sham-controlled trial. *Lancet*. 2021;397:2476–2486.

25. Lee CY, Huang CC, Shih HC, Huang KH. Factors influencing antihypertensive medication compliance in Taiwan: a nationwide population-based study. *Eur J Prev Cardiol*. 2013;20:930–937.

26. Prochnau D, Heymel S, Otto S, Figulla HR, Surber R. Renal denervation with cryoenergy as second-line option is effective in the treatment of resistant hypertension in non-responders to radiofrequency ablation. *EuroIntervention*. 2014;10: 640–645.

27. Balastegui MT, Ramos-Pla JJ, Ferrer-Puchol MD, et al. Cryoplasty versus angioplasty in the treatment of arterial restenosis in an experimental model of atherosclerosis in rabbits. *Cryobiology*. 2015;70:95–100.

28. Tanguay JF, Geoffroy P, Dorval JF, Sirois MG. Percutaneous endoluminal arterial cryoenergy improves vascular remodelling after angioplasty. *Thromb Haemost*. 2004;92:1114–1121.

29. Sarabanda AV, Bunch TJ, Johnson SB, et al. Efficacy and safety of circumferential pulmonary vein isolation using a novel cryothermal balloon ablation system. *J Am Coll Cardiol*. 2005;46:1902–1912.

30. Lee H, Park JH, Floyd JS, Park S, Kim HC. Combined effect of income and medication adherence on mortality in newly treated hypertension: nationwide study of 16 million person-years. *J Am Heart Assoc*. 2019;8:e013148.

31. Wang Y, Wang JW, Wang Y, et al. Monitoring antihypertensive medication adherence by LC-MS/

MS: method establishment and clinical application. *J Cardiovasc Pharmacol*. 2021;78(4):581–596.

32. Liu Y, Zhu B, Zhu L, et al. Thirty-six-month results of laparoscopic-based renal denervation plus unilateral laparoscopic adrenalectomy for the treatment of patients with resistant hypertension caused by unilateral aldosterone-producing adenoma. *J Clin Hypertens (Greenwich)*. 2021;23:946–953.

33. Chen H, Ji M, Zhang Y, et al. Efficiency and safety of renal denervation via cryoablation (Cryo-RDN) in Chinese patients with uncontrolled hypertension: study protocol for a randomized controlled trial. *Trials*. 2019;20:653.

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APPENDIX For a supplemental table, please see the online version of this article.