Catheter-based renal sympathetic denervation induces acute renal inflammation through activation of caspase-1 and NLRP3 inflammasome

Dong Won Lee[1], *Jeong-Su Kim*[1], *Il Young Kim**, *Hyang Sook Kim*[1], *Joo-Young Kim*[1], *Harin Rhee**, *Eun Young Seong**, *Sang Heon Song**, *Soo Bong Lee**, *Charles L. Edelstein*[2], *Ihm Soo Kwak**

Divisions of *Nephrology, and **Cardiology, Department of Internal Medicine, School of Medicine, Pusan National University; Busan-*Republic of Korea*
[1]Research Institute for Convergence of Biomedical Science and Technology, Pusan National University, Yangsan Hospital; Yangsan-*Republic of Korea*
[2]Division of Renal Diseases and Hypertension, University of Colorado Denver; Aurora, Colorado-*USA*

Abstract

Objective: Catheter-based renal sympathetic denervation (RDN) is implemented as a strategy to treat resistant hypertension. Serum creatinine and estimated glomerular filtration rate have some limitations to predict the early stage of acute kidney injury (AKI). We investigated the changes of early inflammatory biomarkers in AKI following the RDN procedure.

Methods: Twenty-five female swine were divided into three groups: normal control (Normal, n=5), sham-operated (Sham, n=5), and RDN groups (RDN, n=15). The RDN group was further subdivided into three subgroups according to the time of sacrifice: immediately (RDN-0, n=5), 1 week (RDN-1, n=5), and 2 weeks (RDN-2, n=5) after RDN. Renal cortical tissue was harvested, and clinical parameters and inflammatory biomarkers were checked.

Results: There were no significant changes in the clinical parameters between the normal control and sham-operated groups using contrast media. Inflammatory interleukin (IL)-1β, IL-18, IL-6, tumor necrosis factor-α, and anti-inflammatory IL-10 increased immediately and then decreased at week 2 after RDN in the renal cortical tissue. Leaderless protein, IL-1α level, increased at week 1 and then decreased at week 2 after RDN. Caspase-1 increased immediately after RDN until week 2. Apoptosis-associated speck-like protein containing a caspase recruitment domain and NLRP3 expressions increased immediately and then decreased at week 2 after RDN.

Conclusion: The RDN could induce acute renal inflammation through the activation of caspase-1 and NLRP3 inflammasome. *(Anatol J Cardiol 2019; 21: 134-41)*

Keywords: acute kidney injury, caspases, hypertension, inflammasomes, renal sympathetic denervation

Introduction

Treatment-resistant hypertension is defined by the American Heart Association as the failure to achieve the target blood pressure (BP) despite the concomitant use of maximally tolerated doses of at least three different antihypertensive agents, including a diuretic (1, 2). The percentage of patients achieving an adequate BP target remains low, (3, 4) thus creating the need for alternative interventional strategies. Endovascular catheter-based radiofrequency renal sympathetic denervation (RDN) has been introduced to denervate efferent and afferent renal sympathetic nerve fibers selectively and implemented as a strategy to treat resistant hypertension (5-7).

Recently, the Symplicity HTN-3 trial reported no further reduction in office or ambulatory BP after 1 year of follow-up (8, 9). However, in the Renal Denervation for Hypertension (DENERHTN) trial, RDN plus standardized stepped-care antihypertensive treatment (SSAHT) showed more decreases in ambulatory BP than the same SSAHT alone at 6 months. This additional BP-lowering effect might contribute to a reduction in cardiovascular morbidity if maintained in the long-term after RDN (10, 11).

Address for correspondence: Dong Won Lee, MD, Division of Nephrology, Department of Internal Medicine, Pusan National University School of Medicine, 20 Geumo-ro Mulgeum-eup Yangsan 50612, Busan-*Republic of Korea*
Phone: 82-10-3275-6499 Fax: 82-55-360-1605 E-mail: dongwonlee@pusan.ac.kr

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Renal function, as assessed by serum creatinine (SCr), estimated glomerular filtration rate (eGFR), and cystatin C concentration in the Symplicity HTN-2 trial, was unchanged from baseline at 6 months after RDN (6). However, in the Symplicity HTN-1 registry, eGFR loss at 36 months was estimated to be 2–8 times lower than that in other contemporary trials including patients at high cardiovascular risk (12, 13). The present study was focused on short-term renal outcomes of inflammatory damage following RDN, especially in the early stage of acute kidney injury (AKI) that lacked any clinical and/or pathological changes. Moreover, traditional clinical parameters, such as SCr and eGFR, have some limitations to predict the early subclinical stage of AKI. Therefore, we investigated whether the RDN procedure might cause any inflammatory damage that induces AKI using early inflammatory biomarkers, such as interleukin (IL)-1β, IL-18, caspase-1, and NLRP3 inflammasome.

Methods

Experimental animal study: Catheter-based RDN in swine

Twenty-five juvenile female swine were used. Their age was 5±0.6 months. The mean weight of the swine was 36.5±1.8 kg. The swine were allowed free access to fresh water and were fed with regular swine chow.

Midazolam 0.5 mg/kg and ketamine 15 mg/kg were administered intravenously, and the trachea was intubated. Ventilation was performed, and general anesthesia was maintained using 1%–2% isoflurane and oxygen. An 8-French introducer sheath was inserted into the right femoral artery using a modified Seldinger's technique (14). Continuous electrocardiogram and BP monitoring were performed. A heparin bolus of 100 U/kg was administered intravenously. An 8-French guiding catheter was inserted to engage each renal artery, and both renal angiograms were obtained using a non-ionic contrast media, iohexol (Omnipaque™, 300 mgI/mL; GE Healthcare, Fairfield, CT, USA, Little Chalfont, UK) 1 mL/kg (0.5 mL/kg each) for the confirmation of normal renal arterial anatomy (15).

Normal control group (Normal) was sacrificed without any procedure. In the sham-operated group using contrast media (Sham), renal denervation catheter was inserted into each renal artery without radiofrequency energy delivery, which was sacrificed immediately after renal angiogram. Actual RDN was performed in the RDN-0, RDN-1, and RDN-2 groups using the Symplicity™ Renal Denervation System (Medtronic, Santa Rosa, CA, USA). The RDN catheter was positioned proximal to the bifurcation of each renal artery, and the impedance of each electrode was checked to identify wall attachment. Radiofrequency ablation was applied consecutively, and a number of either 5 or 6 ablation points were created at each renal artery. The impedance, temperature, and radiofrequency energy delivery were monitored and recorded during the procedure (15). When the RDN procedure was completed, bilateral renal angiograms were obtained again, and signs of renal artery irregularities, such as vasospasm, stenosis, or dissection, were checked.

Clinical parameters

Blood and urine samples were collected at the time of sacrifice by inferior vena cava puncture and urinary bladder puncture, respectively. We measured hemoglobin (Hb), blood urea nitrogen (BUN), SCr, lactate dehydrogenase (LDH), sodium (Na), potassium (K), chloride (Cl), C-reactive protein (CRP), cystatin C, random spot urine protein/creatinine ratio (PCR), and albumin/creatinine ratio (ACR).

Enzyme-linked immunosorbent assay (ELISA)

Renal cortical tissues were harvested at the time of sacrifice and stored at –80°C. Tissue samples were homogenized mechanically (TissueLyser; QAIGEN, Valencia, CA, USA) in a PBS-based buffer containing proteinase inhibitor and non-ionic detergent (Tween 20). Tissue extraction was performed from the supernatant with neutral buffer (PBS with 0.15% Tween 20). IL-1α, IL-1β, IL-18, IL-6, IL-10, and tumor necrosis factor (TNF)-α were determined using a tissue lysate by Porcine Cytokine Magnetic, 6 Plex Kit (Milliplex® MAP, catalog no. PCYTMAG-23K-06; Merck Millipore, Billerica, MA, USA). Caspase-1 activity was measured by Pig caspase-1 ELISA kit (catalog no. CSB-EL004543PI; CUSABIO, Wuhan, China). ELISA was performed according to the manufacturer's instructions.

Immunoblotting

Protein samples were separated on either 8% or 15% SDS-PAGE gels. Gels were transferred to nitrocellulose membranes and blocked with 5% milk protein. Membranes were incubated at 4°C overnight with primary antibodies. Immunoblot analyses of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing (NLRP) were performed using the following primary antibodies: (1) purified rabbit anti-ASC polyclonal antibody (1:1000) (catalog no. bs-6741R; Bioss, Woburn, MA, USA) and (2) goat anti-human NLRP3 polyclonal antibody (1:200) (catalog no. MBS241660; MyBioSource, San Diego, CA, USA).

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). Non-normally distributed data were analyzed by the non-parametric Kruskal–Wallis H test. The Mann–Whitney U test was performed and then corrected by Bonferroni test for post-hoc test. A p-value of <0.05 was considered statistically significant. Values were expressed as mean±standard error.

Ethics statement

The procedures used and the care of the animals complied with the Guides for the Care and Use of Laboratory Animals

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published by the United States National Institute of Health (publication no. 85-23, revised 1996). The experiment was performed at the Preclinical Trial and Training Center (Pusan National University, Yangsan Hospital, Yangsan, Korea) and was approved by the Institutional Animal Care and Use Committee in Pusan National University Yangsan Hospital (approval no. 2014-020).

Results

Clinical parameters

There were no significant changes between the normal control and sham-operated groups. Serum Hb, BUN, creatinine, cystatin C, Na, K, Cl, CRP, and random spot urine PCR and ACR showed no significant differences between the groups (Table 1 and Fig. 1a, 1b).

Serum LDH levels increased immediately after RDN (p=0.035, Normal vs. RDN-0 and p=0.134, RDN-0 vs. RDN-1) and then decreased at week 2 (p=0.024, RDN-1 vs. RDN-2) (Fig. 1c).

Inflammatory responses in the renal cortex

Leaderless protein, IL-1α level, increased at week 1 (p=0.021, Normal vs. RDN-1) and then decreased at week 2 after RDN (p=0.025, RDN-1 vs. RDN-2) (Table 2 and Fig. 2a). Proinflammatory cytokines, IL-1β and IL-18 levels, increased immediately after RDN (p=0.012 and p=0.032, Normal vs. RDN-0 and p=0.028 and p=0.045, RDN-0 vs. RDN-1, respectively) and then decreased

Table 1. Clinical parameters after RDN

	Normal	Sham	RDN-0	RDN-1	RDN-2
Hb (g/dL)	10.3±0.5	9.7±0.2	9.6±0.3	11.4±1.4	10.5±1.1
BUN (mg/dL)	6.4±0.4	7.1±1.7	9.0±0.5	6.6±0.7	8.9±1.1
SCr (mg/dL)	0.9±0.1	0.9±0.0	1.1±0.0	1.3±0.0	1.2±0.0
LDH (U/L)	745.3±24.1	796.3±46.1	1159.7±132.3[a]	1131.0±166.7[a]	836.0±18.9[b]
Na (mEq/L)	142.3±1.5	140.3±0.9	142.0±0.0	143.3±0.9	142.3±0.3
K (mEq/L)	4.0±0.2	3.8±0.2	4.3±0.1	4.5±0.3	4.5±0.4
Cl (mEq/L)	102.7±0.9	101.3±0.9	103.0±0.6	100.3±1.3	98.0±1.5
CRP (mg/L)	0.09±0.04	0.02±0.00	0.09±0.01	0.02±0.00	0.03±0.01
CysC (mg/L)	0.00±0.00	0.00±0.00	0.17±0.09	0.27±0.03	0.27±0.03
UPCR (mg/g)	154.5±16.7	153.8±1.5	188.8±9.7	150.4±22.8	118.9±17.3
UACR (mg/g)	7.5±3.3	3.5±1.2	3.1±1.1	4.0±1.8	2.4±0.3

[a] $P<0.05$, Normal.
[b] $P<0.05$, RDN-1.
Normal - normal control group; Sham - sham-operated group using contrast media; RDN-0 - renal sympathetic denervation (RDN) group sacrificed immediately after RDN; RDN-1 - RDN group sacrificed 1 week after RDN; RDN-2 - RDN group sacrificed 2 weeks after RDN; Hb - hemoglobin; BUN - blood urea nitrogen; SCr - serum creatinine; LDH - lactate dehydrogenase; CRP - C-reactive protein; CysC - cystatin C; UPCR - random spot urine protein/creatinine ratio; UACR - random spot urine albumin/creatinine ratio

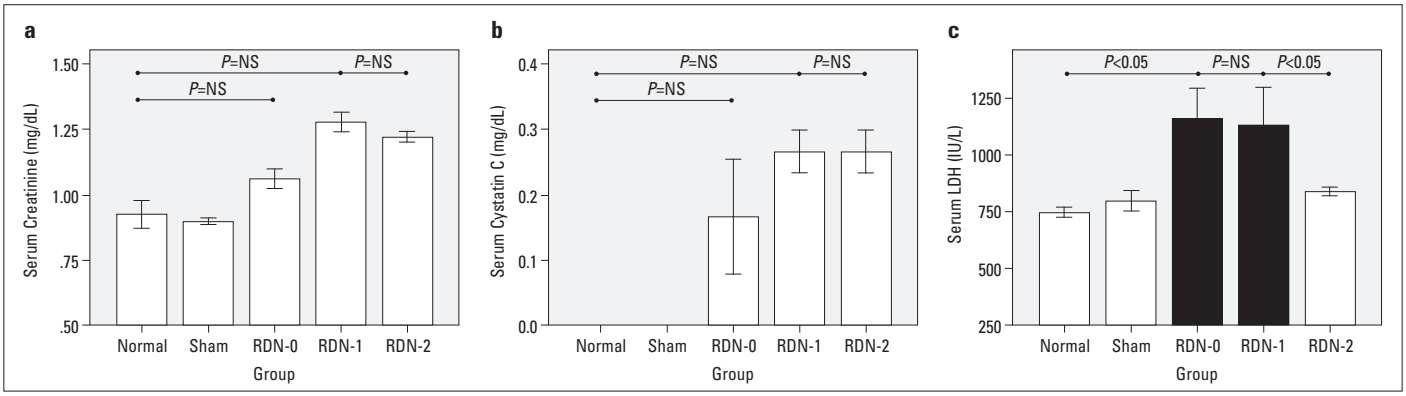

Figure 1. Induction of AKI after RDN. (a) Serum creatinine and (b) cystatin C showed a tendency to increase from week 1 to 2 after RDN (P=NS, Normal vs. RDN-1 and Normal vs. RDN-2, respectively). (c) Serum LDH levels increased (P=0.035, Normal vs. RDN-0 and P=0.134, RDN-0 vs. RDN-1) and decreased at week 2 after RDN (P=0.024, RDN-1 vs. RDN-2).
Normal - normal control group; Sham - sham-operated group using contrast media; RDN-0 - renal sympathetic denervation (RDN) group sacrificed immediately after RDN; RDN-1 - RDN group sacrificed 1 week after RDN; RDN-2 - RDN group sacrificed 2 weeks after RDN; AKI - acute kidney injury; LDH - lactate dehydrogenase

Table 2. Cytokines after RDN

	Normal	Sham	RDN-0	RDN-1	RDN-2
IL-1α (pg/mg)	1.6±0.1	1.7±0.2	2.1±0.1	3.5±0.3[a]	2.2±0.1[b]
IL-1β (pg/mg)	2.9±1.1	3.5±0.4	15.2±1.0[c]	19.7±1.9[d]	6.5±0.6[b]
IL-18 (pg/mg)	825.3±38.2	913.3±44.8	1102.3±16.0[c]	1256.3±54.0[d]	855.6±64.7[b]
IL-6 (pg/mg)	0.9±0.5	1.4±0.4	2.9±0.5[c]	3.7±0.3[d]	2.5±0.2[b]
TNFα (pg/mg)	24.8±6.7	42.7±9.2	62.0±4.8[c]	85.4±11.7	41.1±11.7[b]
IL-10 (pg/mg)	9.0±2.1	14.6±2.6	22.7±0.7[c]	35.1±5.0[d]	18.8±0.2[b]
Casp-1 (mmol/mg)	1.2±0.0	1.3±0.1	1.7±0.0[c]	2.0±0.2[d]	1.7±0.1

[a]*P*<0.05, Normal/Sham/RDN-0.
[b]*P*<0.05, RDN-1.
[c]*P*<0.05, Normal/Sham.
[d]*P*<0.05, RDN-0.
Normal - normal control group; Sham - sham-operated group using contrast media; RDN-0 - renal sympathetic denervation (RDN) group sacrificed immediately after RDN; RDN-1 - RDN group sacrificed 1 week after RDN; RDN-2 - RDN group sacrificed 2 weeks after RDN; Casp-1 - caspase-1

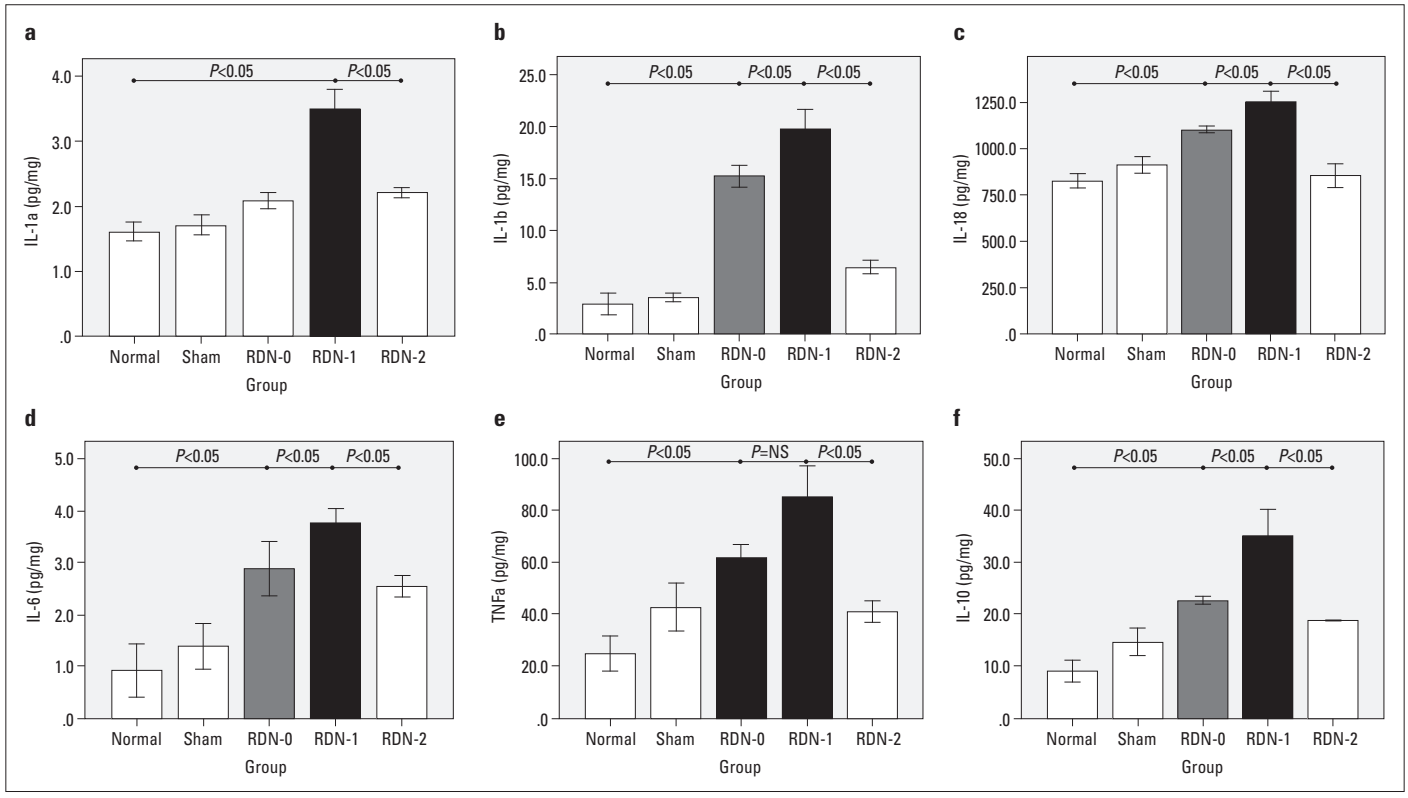

Figure 2. Proinflammatory and anti-inflammatory cytokines in AKI after RDN. (a) IL-1α level increased at week 1 (*P*=0.021, Normal vs. RDN-1) and then decreased at week 2 after RDN (*P*=0.025, RDN-1 vs. RDN-2). (b, c) IL-1β and IL-18 levels increased (*P*=0.012 and *P*=0.032, Normal vs. RDN-0 and *P*=0.028 and *P*=0.045, RDN-0 vs. RDN-1, respectively) and then decreased at week 2 after RDN (*P*=0.018 and *P*=0.023, RDN-1 vs. RDN-2, respectively). (d, e) IL-6 and TNF-α levels increased (*P*=0.021 and *P*=0.023, Normal vs. RDN-0 and *P*=0.041 and *P*=0.127, RDN-0 vs. RDN-1, respectively) and then decreased at week 2 after RDN (*P*=0.025 and *P*=0.016, RDN-1 vs. RDN-2, respectively). (f) IL-10 level increased (*P*=0.031, Normal vs. RDN-0 and *P*=0.028, RDN-0 vs. RDN-1, respectively) and then decreased at week 2 after RDN (*P*=0.016, RDN-1 vs. RDN-2).
Normal - normal control group; RDN-0 - renal sympathetic denervation (RDN) group sacrificed immediately after RDN; RDN-1 - RDN group sacrificed 1 week after RDN; RDN-2 - RDN group sacrificed 2 weeks after RDN; AKI - acute kidney injury; IL - interleukin; TNF - tumor necrosis factor

at week 2 after RDN (p=0.018 and p=0.023, RDN-1 vs. RDN-2, respectively) (Table 2 and Fig. 2b, 2c). Inflammatory cytokines, IL-6 and TNF-α levels, increased immediately after RDN (p=0.021 and p=0.023, Normal vs. RDN-0 and p=0.041 and p=0.127, RDN-0 vs. RDN-1, respectively) and then decreased at week 2 (p=0.025 and p=0.016, RDN-1 vs. RDN-2, respectively) (Table 2 and Fig. 2d, 2e). Anti-inflammatory cytokine, IL-10 level, increased immediately after RDN (p=0.031, Normal vs. RDN-0 and p=0.028, RDN-0 vs.

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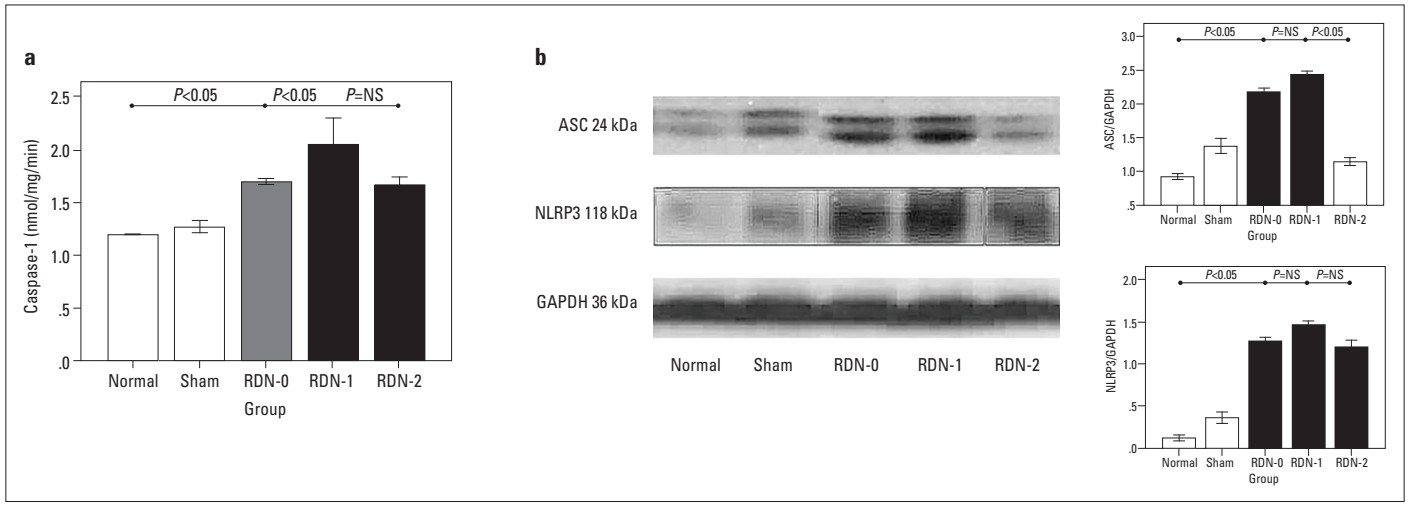

Figure 3. Renal ASC, NLRP3, and caspase-1 in AKI after RDN. (a) Caspase-1 activity increased continuously after RDN (*P*=0.028, Normal vs. RDN-0; *P*=0.034, RDN-0 vs. RDN-1; and *P*=0.056, RDN-1 vs. RDN-2). (b) Renal ASC (24 kDa) expression increased (*P*=0.013, Normal vs. RDN-0 and *P*=0.120, RDN-0 vs. RDN-1) and then decreased at week 2 after RDN (P=0.010, RDN-1 vs. RDN-2). Renal NLRP3 (118 kDa) expression increased continuously after RDN (*P*=0.016, Normal vs. RDN-0; *P*=0.120, RDN-0 vs. RDN-1; and *P*=0.064, RDN-1 vs. RDN-2). In the densitometric analysis of immunoblots, data are presented as protein/GAPDH ratios plotted on the y-axis. GAPDH (36 kDa) was used as the loading control and did not vary among the groups. Representative immunoblots of at least three separate experiments.

Normal - normal control group; RDN-0 - renal sympathetic denervation (RDN) group sacrificed immediately after RDN; RDN-1 - RDN group sacrificed 1 week after RDN; RDN-2 - RDN group sacrificed 2 weeks after RDN; ASC - apoptosis-associated speck-like protein containing a caspase recruitment domain; AKI - acute kidney injury; GAPDH - glyceraldehyde 3-phosphate dehydrogenase

RDN-1, respectively) and then decreased at week 2 (p=0.016, RDN-1 vs. RDN-2) (Table 2 and Fig. 2f).

Acute renal inflammation through the activation of NLRP3 inflammasome

Caspase-1 activity increased immediately and continuously after RDN (p=0.028, Normal vs. RDN-0; p=0.034, RDN-0 vs. RDN-1; and p=0.056, RDN-1 vs. RDN-2) (Table 2 and Fig. 3a). Renal ASC expression increased immediately after RDN (p=0.013, Normal vs. RDN-0 and p=0.120, RDN-0 vs. RDN-1) and then decreased at week 2 (p=0.010, RDN-1 vs. RDN-2). NLRP3 expression also increased immediately after RDN (p=0.016, Normal vs. RDN-0 and p=0.120, RDN-0 vs. RDN-1) and then showed a tendency to decrease at week 2 (p=0.064, RDN-1 vs. RDN-2) (Fig. 3b).

Discussion

Renal somatic afferent nerves to central sympathetic drive and efferent sympathetic signaling to the kidneys are closely related to the development of hypertension, heart failure, and chronic kidney disease (16, 17). Experimental studies have demonstrated that renal sympathetic nerve activation enhances noradrenaline production or spillover, whereas renal denervation results in a marked decrease of noradrenaline by up to 95% (18-21). Minimally invasive RDN has emerged as an effective therapy for resistant hypertension. However, the long-term safety and efficacy of RDN are still under investigation. The Symplicity HTN-1 and HTN-2 trials demonstrated the renal safety of RDN as assessed by clinical parameters, such as SCr, eGFR, and cystatin C (5, 6), suggesting the need for *in vitro* experiments with inflammatory biomarkers in the early stage of AKI. Therefore, we hypothesized that the RDN procedure might cause subclinical AKI. To test this hypothesis, in the present study, we evaluated the early inflammatory response after RDN using inflammatory biomarkers, such as IL-1β, IL-18, caspase-1, and NLRP3 inflammasome.

Recognition of the injurious role of inflammation in AKI is increasing and is accompanied by the involvement of leukocytes, adhesion molecules, and cytokines (22-25). The inflammasome is a molecular complex that contains NLRP proteins and an adaptor protein, ASC (26, 27). The most fully characterized inflammasome is the NLRP3 inflammasome that contains the NLRP3 protein (28). Proinflammatory caspase-1, which is activated by inflammasome complexes in response to pathogen-associated molecular patterns and damage-associated molecular patterns, converts IL-1β and IL-18 to their active forms (29, 30). The inflammasome is activated mainly in the inflammatory cells, where it plays an important role in the innate immune response, and causes tissue inflammation and apoptosis (27, 28). Caspase-1 is a mediator of both cisplatin-induced (31) and ischemic (32) AKI. Previously, we demonstrated that a pan-caspase inhibitor decreased caspase-1, IL-1α, and IL-1β levels and protected against necrosis of cisplatin-induced AKI (33). In addition, NLRP3 inflammasome inhibition (knockout) protects against ischemic AKI (34). In the present study, the levels of proinflammatory cytokines, IL-1β and IL-18, inflammatory cytokines, IL-6 and TNF-α, and anti-inflammatory cytokine, IL-10, increased and then recovered

in the kidney at week 2 after RDN. IL-1β-converting enzyme, caspase-1 activity, increased, and ASC and NLRP3 expressions also increased in the kidney, suggesting a self-limited inflammatory response to the RDN procedure. However, there were no significant changes in traditional clinical parameters among the groups. Although the changes in early inflammatory biomarkers did not imply clinical and histological damages, we should, at least, take strict precautions to protect against subclinical AKI after RDN. In a recent animal study, they used an experimental method of stripping the sheath and adventitia from the exposed left renal artery and vein to destroy the unilateral sympathetic nerve fibers in the renal ischemia/reperfusion injury rat model and demonstrated that renal denervation could relieve long-term sequelae of ischemic renal injury, such as interstitial inflammation, fibrosis, and oxidative stress (35). The sympathetic stripping was different from the catheter-based RDN in our study because it was a mechanical, non-selective block of the unilateral sympathetic nerve fiber. In our study, the RDN performed on pigs was the same procedure applied to humans, and the sympathetic nerve fibers of both sides were selectively cauterized via intravascular catheter and probe. Our study was to evaluate the renal safety of the RDN procedure, especially in the absence of concurrent acute or chronic renal impairment. We tried to identify the preceding inflammatory response caused by the RDN procedure itself when applied to normal pigs without acute or chronic kidney injury. Further research is needed to determine whether these potential inflammatory responses may be risk factors for the future expression of clinical AKI, and whether such damage can be prevented by inhibiting the inflammatory mediators.

Although the Symplicity HTN-3 trial supported no further reduction in office or ambulatory BP after 1 year of follow-up, (8, 9) this failure did not suggest that the RDN should be abandoned. The DENERHTN trial showed that RDN plus SSAHT could also decrease ambulatory BP at 6 months of follow-up (10). Therefore the RDN might contribute to an improvement in renal and cardiovascular morbidity.

Recently, the SPYRAL HTN-OFF MED and SPYRAL HTN-ON MED studies provided biological proof of principle for the BP-lowering efficacy of RDN compared with sham control with no major safety events (36, 37). In addition, the RADIANCE-HTN SOLO trial showed a safer alternative to radiofrequency ablation and a proof-of-concept data for the Paradise endovascular ultrasound renal denervation system (38). However, those trials showed renal safety profile only with traditional renal markers, such as SCr and eGFR. Therefore, our animal study using early inflammatory biomarkers is a unique experiment, suggesting the risk of early inflammatory AKI.

Study limitations

Our study has several limitations. This was a small, experimental animal study. However, the RDN procedures were performed successfully by an expert with an experience in endovascular procedures including RDN to human patients. The impedance of each electrode, temperature, and radiofrequency energy delivery was monitored during the procedure. Post-RDN renal angiograms were obtained again, and the appropriateness of the procedure was confirmed. The present study needs more histological and immunohistochemical data to explain the site of inflammation. However, some studies have shown no structural renal damage after RDN as assessed by magnetic resonance imaging and histology (39, 40). Moreover, traditional clinical parameters, such as BUN and SCr, have some limitations to predict the early stage of AKI. Therefore, we investigated the early inflammatory changes, especially in the stage of potential AKI that lacked any clinical and/or histological changes. This experiment was focused on short-term renal outcomes of AKI following RDN. The increases of those inflammatory biomarkers reflect subclinical AKI after RDN. Finally, these results suggest that RDN might cause acute renal inflammation through the activation of caspase-1 and NLRP3 inflammasome. However, no hemodynamic data of BP or catecholamines were provided. We did not use hypertensive animal model and aimed to evaluate the safety of the RDN procedure itself.

Conclusion

The RDN procedure has been known to be safe with regard to traditional renal surrogate markers, such as SCr and eGFR. The present study showed that the RDN procedure could cause acute renal inflammation through the activation of caspase-1 and NLRP3 inflammasome.

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