Establishment of a model of atrial fibrillation associated with chronic kidney disease in rats and the role of oxidative stress

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BACKGROUND An animal model of atrial fibrillation (AF) associated with chronic kidney disease (CKD) has not been available.

OBJECTIVE The purpose of this study was to test the validity of 5/6 nephrectomy (5.6Nx) as an appropriate model of AF associated with CKD and to investigate the role of oxidative stress.

METHODS Male Sprague-Dawley rats were subjected to 5.6Nx. A novel derivative of lipoic acid, sodium zinc dihydrolipoylhistidinate (DHLHZn), was subcutaneously infused. Four weeks later, hearts were isolated.

RESULTS We observed 5 main findings. (1) 5.6Nx induced renal dysfunction with elevation of systolic blood pressure and impaired glucose tolerance. (2) In the left atrium (LA), expressions of α-smooth muscle action and collagen type I, the compositional proteins of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and malondialdehyde were increased by 5.6Nx, which was reversed by DHLHZn treatment. (3) In the LA, the tissue content of angiotensin II was elevated by 5.6Nx, which was also reversed by DHLHZn. (4) Masson trichrome staining revealed that heterogeneous LA interstitial fibrosis was induced by 5.6Nx, which was attenuated by DHLHZn. (5) In isolated perfused heart experiments, 5.6Nx caused slowing of interatrial conduction. In the hearts of rats of the 5.6Nxgroup, right atrial extrastimuli invariably induced AF (8/8 [100%]), which were suppressed by DHLHZn (3/8 [38%], $P < .05$).

CONCLUSION We successfully established an appropriate model of AF associated with CKD in rats. Because the amount of NADPH oxidase was increased and the potent antioxidant agent DHLHZn was effective, oxidative stress may be involved in the pathogenesis of LA fibrosis and enhanced AF vulnerability in our model.

KEYWORDS Atrial fibrillation; Chronic kidney disease; Nephrectomy; Oxidative stress

ABBREVIATIONS **AF** = atrial fibrillation; **CKD** = chronic kidney disease; **CL** = cycle length; **DHLHZn** = sodium zinc dihydrolipoylhistidinate; **ERP** = effective refractory period; **GAPDH** = glyceraldehyde-3-phosphate dehydrogenase; **hs-CRP** = high-sensitivity C-reactive protein; **IPGTT** = intraperitoneal glucose tolerance test; **LA** = left atrium; **LV** = left ventricle; **MDA** = malondialdehyde; **NADPH** = nicotinamide adenine dinucleotide phosphate; **Nx** = nephrectomy; **RA** = right atrium; **SBP** = systolic blood pressure; **SDS-PAGE** = sodium dodecyl sulfate–polyacrylamide gel electrophoresis; **SMA** = smooth muscle actin; **VEH** = vehicle

(Heart Rhythm 2012;9:2023–2031)

Introduction

Atrial fibrillation (AF) is the most common arrhythmia seen and is associated with significant morbidity and mortality.[1,2] Recent studies have revealed the close relationship between AF and chronic kidney disease (CKD) in patients not on dialysis.[3–6] Analysis based on the Chronic Renal Insufficiency Cohort (CRIC) study indicated that the prevalence of AF was 18% in participants with mild-to-moderate CKD (mean age 58.6 years, mean estimated glomerular filtration rate 43.6 mL/min), suggesting that the prevalence of AF was 2- to 3-fold higher than that of the general population.[3] More recently, it was demonstrated that, compared with participants without CKD, the odds ratios for the prevalence of AF were 2.67, 1.68, and 3.52 among those with stage 1–2, stage 3, and stage 4–5 CKD, respectively.[6] Therefore, elucidating the mechanisms responsible for the development of AF among CKD patients and exploring preventive strategies is urgent. Few studies investigating the pathogenesis of CKD-related AF have been performed because an appropriate animal model has not been available.

The 5/6 nephrectomized (5.6Nx) rats have been used widely to represent the pathogenesis of CKD in humans.[7] In 5.6Nx rats, a major contributor to the elevation in circulating levels of inflammatory biomarkers may be enhanced oxidative stress, the mechanisms of which involve activation of nicotinamide adenine dinucleotide phosphate-oxidase s(NADPH) oxidase.[8,9] Sodium zinc dihydrolipoylhis-

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1547-5271/$ -see front matter http://dx.doi.org/10.1016/j.hrthm.2012.08.019

tidinate (DHLHZn) is a new Zn^{2+}/dihydrolipoic acid derivate complex. It is based on lipoic acid and histidine and thus possesses antioxidant effects.[10–12] In neonatal cultured cardiomyocytes, we demonstrated that H_2O_2-induced increase in levels of reactive oxygen species was attenuated by a 1-hour pretreatment with DHLHZn.[12]

In this study, we tested the hypothesis that atrial fibrosis and enhanced AF vulnerability could be induced in the hearts of 5.6Nx rats and investigated the role of oxidative stress using DHLHZn.

Methods

All experimental procedures were conducted in accordance with the guidelines set by the Physiological Society of Oita University (Oita, Japan) for the care and use of laboratory animals. Those guidelines followed the guidelines established by the National Institutes of Health (Bethesda, MD).

Animals, surgical procedures, and DHLHZn administration

Male, 8-week-old Sprague-Dawley rats were used. The 5.6Nx group underwent nephrectomy by resection of the upper and lower thirds of the left kidney, followed by right nephrectomy 7 days later (5.6Nx group).[7–9] The control group underwent a sham procedure (Sham group). Procedures were performed with the animals under general anesthesia (pentobarbital sodium 50 mg/kg IP). One day after the completion of nephrectomy, rats were randomized to a group treated with either vehicle (VEH group) or DHLHZn (DHL group). An osmotic minipump (Alzet 2ML4, Alzet, Cupertino, CA) was implanted subcutaneously into rats for constant infusion of DHLHZn (5 mg/kg/day) or vehicle. DHLHZn was dissolved in physiologic (0.9%) saline. DHLHZn was kindly donated by Oga Research (Osaka, Japan).

Isolation and culture of adult rat atrial cardiac fibroblasts

Atrial cardiac fibroblasts were isolated from male, 6-week-old Sprague-Dawley rats. The left atrium (LA) was removed and minced in phosphate-buffered saline. The methodologic details of the isolation and culture of cells is described elsewhere.[13] Fibroblasts at passage 2 were used. Angiotensin II (100 nmol/L) was added to the medium for 24 hours. Pretreatment with DHLHZn (10 mmol/L) was started 1 hour before application of angiotensin II. Thereafter, fibroblasts were collected for evaluation of the expression of α-smooth muscle actin (α-SMA).

Blood pressure and echocardiography

Systolic blood pressure (SBP) was measured periodically by the noninvasive tail-cuff method (MK-1000, Muromachi Kikai Co, Ltd, Tokyo, Japan). Transthoracic echocardiography (Aloka, Tokyo, Japan) was performed at day 28. Left atrial dimension, interventricular septal wall thickness, left ventricular (LV) posterior wall thickness, LV end-diastolic dimension, LV end-systolic dimension, and LV ejection fraction were determined.

Intraperitoneal glucose tolerance test

At day 28, the intraperitoneal glucose tolerance test (IPGTT) was undertaken after an overnight fast.[14] Glucose solution (2 g/kg) was administered via the intraperitoneal route. Blood was drawn from the tail vain at 0, 15, 30, 60, and 120 minutes.

Western blot analysis

Frozen LA tissues were homogenized and centrifuged. Protein concentrations were measured by the Bradford method.[13,15] An equal amount of total protein was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred by electrophoresis onto a polyvinyl-idenedifluoride (PVDF) membrane (Bio-Rad, Hercules, CA). After blocking, membranes were incubated with mouse anti-rat α-SMA antibody (Sigma-Aldrich, St. Louis, MO), goat anti-rat collagen type I antibody (Santa Cruz Biotechnology, Santa Cruz, CA), goat anti-rat anti-$p47^{phox}$ antibody, goat anti-rat anti-Nox4, and anti-goat anti-rat anti-$gp91^{phox}$ antibody (Santa Cruz Biotechnology), rabbit anti-rat malondialdehyde (MDA) antibody (Academy Bio-Medical, Houston, TX, USA), or rabbit anti-rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Sigma-Aldrich). After washing, membranes were incubated with horseradish peroxidase–tagged secondary antibodies (GE Healthcare, Amersham, UK). Anti-rat α-SMA antibody was also used for Western blotting using cultured atrial fibroblasts.

Angiotensin II concentration assay

The angiotensin II contents of LA tissues were measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals Inc, Saint Joseph, MO).[16]

Histologic studies

LA and LV tissues were carefully removed, fixed in 10% phosphate-buffered formalin, and embedded in paraffin. Deparaffined sections (5-μm thickness) were stained with Masson trichrome. Microscopic images were scanned into a personal computer using Photoshop 7.0 (Adobe, San Jose, CA). Connective tissue was differentiated on the basis of its color and expressed as a percentage of the reference tissue area using Scion image software (Scion, Frederick, MD). In each atria or ventricle, 3 images with a magnification of $\times$400 were analyzed and averaged.

Electrophysiologic studies

On day 28, hearts were isolated and perfused in retrograde fashion using a Langendorff apparatus with Krebs-Henseleit buffer equilibrated with a 95% O_2/5% CO_2 gas mixture at 36.5°C and at a constant pressure of 75 mm Hg. Two sets of Teflon-coated (except their tips) silver bipolar electrodes, each with an interelectrode distance of 10 mm, were placed on the appendages of the right atrium (RA) and LA. The effective refractory period (ERP) of the LA was measured by the S2 extrastimulus method using 8 regularly paced beats with cycle lengths (CL) of 200, 150, 120, and 90 ms.