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Basic Research

Importance of Pulmonary Vein Preferential Fibrosis for Atrial Fibrillation Promotion in Hypertensive Rat Hearts

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ABSTRACT

Background: Hypertension is one of the independent risk factors for atrial fibrillation (AF). Pulmonary veins (PVs) play an important role as the substrate for AF and triggers of AF. The purpose of this study was to determine the structural remodelling of the PVs and its effect on promoting AF in hypertensive (HT) rat hearts.

Methods: Eighteen-week-old Dahl salt-sensitive HT rats and their controls were used for histological and immunohistological analyses, and electrophysiological studies were performed in Langendorff perfused hearts.

Results: Masson-trichrome staining revealed that hypertension significantly increased the fibrosis in the PVs, particularly in subendocardial and perivascular areas, compared with that in control rats, however, at this early stage of hypertension, left atrial fibrosis was not prominent. In the HT rat hearts with PVs, electrical stimulation significantly increased the number of repetitive atrial firing and atrial

RÉSUMÉ

Introduction : L'hypertension constitue un des facteurs de risque indépendants de la fibrillation auriculaire (FA). Les veines pulmonaires jouent un rôle important à titre de substrat et de déclencheur de la FA. L'objectif de cette étude était de déterminer si le remodelage structurel des veines pulmonaires avait une incidence sur l'apparition d'une FA chez des rats hypertendus.

Méthodes : Des rats Dahl sensibles au sel et hypertendus âgés de 18 semaines ainsi que leurs témoins ont fait l'objet d'analyses histologiques et immunohistologiques, tandis que des études électrophysiologiques ont été menées sur des cœurs isolés perfusés selon la technique de Langendorff.

Résultats : Une coloration trichromique de Masson a révélé que l'hypertension augmentait de manière significative la fibrose des veines pulmonaires, particulièrement dans les régions sous-endocardique et périvasculaire, comparativement aux rats témoins, mais qu'à ce stade

Hypertension (HT) is one of the independent risk factors for atrial fibrillation (AF).[1] The exact mechanisms of AF in patients with HT remains to be determined. Experimental studies with various HT models have suggested that HT-induced atrial structural remodelling plays a crucial role in the promotion and perpetuation of AF,[2-4] as shown in clinical studies that used cardiac magnetic resonance imaging.[5] Most of the experimental studies focused on structural remodelling including fibrosis and connexin expression[3,6,7] in atria, which should be one of the important components of AF perpetuation. Recently, it has become well known that the pulmonary veins (PVs) in patients with AF exhibit marked discontinuity and fibrosis.[8] Those results suggest that the structural changes in the PVs might also play an important role in the development of AF and provide an important source of ectopic beats, which can initiate AF.[9] Nevertheless, little is known about the precise mechanisms of the development of PV structural remodelling in HT. The aim of this study was to elucidate the characteristics of PV fibrosis in HT using a Dahl salt-sensitive rat model.

Methods

Animal model

Male Dahl salt-sensitive rats were obtained from Japan SLC Inc (Hamamatsu, Japan). The rats were randomly divided into 3 groups at 6-weeks-old: normal salt (0.3% NaCl; control [CONT] group), high salt (8% NaCl; HT group), and high salt (8% NaCl) with imatinib treatment (HT + IMA group). Treatment with imatinib mesylate, a PDGFR activity blocker (ALX-270-492, Enzo Life Science), was started at a dose of 20 mg/kg/d from 16 weeks of age for

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tachycardia inducibility, which significantly diminished after the excision of the PVs. An immunofluorescent analysis revealed that HT rats had PV specific endocardial smooth muscle actin (αSMA)-positive cells with remarkable proliferation of platelet-derived growth factor (PDGF)-C and vascular endothelial growth factor (VEGF), which was lacking in the left atrial structures of the control and the HT rats. Pretreatment with imatinib, a PDGF receptor activity blocker, in HT rats reduced the αSMA-positive cell proliferation and fibrosis in the PVs and also induced a significant reduction in VEGF expression. Also, the drug pretreatment effectively prevented repetitive atrial firing promotion without affecting the blood pressure.

Conclusions: PV preferential fibrosis might play an important role in the arrhythmogenic substrate of AF in HT rat hearts.

précoce de l'hypertension la fibrose auriculaire gauche n'était pas très marquée. Une stimulation électrique des cœurs de rats hypertendus avec les veines pulmonaires a significativement accru le nombre d'impulsions répétitives aux oreillettes et la capacité à induire la tachycardie auriculaire, lesquels ont été réduits de manière significative suivant l'excision des veines pulmonaires. Une analyse par immunofluorescence a révélé que les rats hypertendus avaient des cellules positives pour l'actine musculaire lisse alpha (αSMA+, de l'anglais *α-smooth muscle actin*) endocardiques dans les veines pulmonaires, avec une augmentation très importante de l'expression du facteur de croissance dérivé des plaquettes C (PDGF-C, de l'anglais *platelet-derived growth factor C*) et du facteur de croissance endothéliale vasculaire (VEGF, de l'anglais *vascular endothelial growth factor*), ce qui n'était pas le cas des structures auriculaires gauches des rats témoins et des rats hypertendus. Un traitement préalable par l'imatinib, un inhibiteur de l'activité du récepteur du PDGF, chez les rats hypertendus a permis de réduire la prolifération des cellules αSMA+ et la fibrose des veines pulmonaires, en plus de réduire de manière significative l'expression du VEGF. Ce traitement a aussi permis de prévenir efficacement le nombre d'impulsions répétitives aux oreillettes sans incidence sur la pression artérielle.

Conclusions : Une fibrose des veines pulmonaires pourrait jouer un rôle important à titre de substrat arythmogène de la FA chez les rats hypertendus.

2 weeks in the HT rat group (Fig. 1A). The systolic blood pressure and heart rate were noninvasively measured as previously reported[10] using the tail-cuff method (BP-98A; Softron, Tokyo, Japan) every 2 weeks from 6 weeks of age.

The rats were killed at 18 weeks of age for a histological and immunofluorescence analysis, and electrophysiological study. The experimental protocol was approved by the animal ethical committee of the Cardiovascular Institute (Tokyo, Japan).

Histology

A horizontal section 8-μm thick was stained with Masson-trichrome to evaluate the fibrotic area in the left atrium (LA) and PVs. Images were acquired with a digital microscope, (COOLSCOPE II; Nikon, Tokyo, Japan). To quantify the fibrotic area in the digitalized photos, aniline blue areas were measured relative to the total tissue area using Image Pro Discovery 4.5 (Media Cybernetics) image analysis software. The mean value was obtained from 10 blindly-selected different fields for each rat.

Immunofluorescence staining

Frozen sections (8-μm thickness) fixed in acetone were incubated with the primary antibodies listed in Supplemental Table S1 (CD31, αSMA, heat shock protein 47 [HSP47], H-Caldesmon, VEGF, and PDGF-C). Immunofluorescent staining with Alexa Fluor 488 or 568 conjugated goat anti-rabbit antibodies or goat anti-mouse antibodies for microscopy was performed. The cell nuclei were stained with DAPI (4′,6-Diamidino-2-Phenylindole, Dilactate). Immunofluorescence-labelled sections were examined with a Pascal Zeiss laser scanning microscope. The green channel had an excitation of 488 nm and an emission of 525 nm. The red channel had and excitation of 596 nm and an emission of 620 nm. A lack of any crosstalk between the channels was established. Immunofluorescent images were quantified using magnification ×100 with an AxioVision digital imaging program (Carl Zeiss, Version 4.8.2).

Western blot analysis

Total protein samples (10 μg) extracted from the left atrial appendage (LAA) were separated using 8% polyacrylamide sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Amersham Hybond-P). The PVDF membranes were incubated with primary antibodies of anti-PDGF-C and anti-VEGF (Supplemental Table S1), and subsequently incubated with gout anti-rabbit immunoglobulin G conjugated to horseradish peroxidase. All expression data were relative to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) staining for the same samples on the same gels.

Electrophysiological study

To determine whether HT causes arrhythmogenic substrates of atrial tachyarrhythmias, an electrophysiological study was performed using Langendorff perfused hearts as previously reported.[10,11] Briefly, the hearts with parts of the lungs including the PVs at 18 weeks of age were rapidly removed and connected to the Langendorff apparatus perfused with Tyrode's solution containing (mmol/L) NaCl 136.5, KCl 5.4, HEPES (4-[2-HydroxyEthyl)]-1-PiperazineEthaneSulfonic acid) 5.5, Na_2HPO4 0.33, glucose 5.5, $CaCl_2$ 1.8, and $MgCl_2$ 0.53 (pH 7.4 adjusted with NaOH). Two bipolar electrodes were attached to the LAA and right atrial appendage for pacing and recording, respectively (Fig. 2A). After 20 minutes of recovery in sinus rhythm, single extrastimulus pacing at a basic cycle length of 200 ms was delivered from the LAA. The stimulation was performed with 1-ms rectangular