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Original article

Histone-deacetylase inhibition reverses atrial arrhythmia inducibility and fibrosis in cardiac hypertrophy independent of angiotensin

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ABSTRACT

Atrial fibrosis influences the development of atrial fibrillation (AF), particularly in the setting of structural heart disease where angiotensin-inhibition is partially effective for reducing atrial fibrosis and AF. Histone-deacetylase inhibition reduces cardiac hypertrophy and fibrosis, so we sought to determine if the HDAC inhibitor trichostatin A (TSA) could reduce atrial fibrosis and arrhythmias. Mice over-expressing homeodomain-only protein ($HopX^{Tg}$), which recruits HDAC activity to induce cardiac hypertrophy were investigated in 4 groups (aged 14–18 weeks): wild-type (WT), $HopX^{Tg}$, $HopX^{Tg}$ mice treated with TSA for 2 weeks (TSA-HopX) and wild-type mice treated with TSA for 2 weeks (TSA-WT). These groups were characterized using invasive electrophysiology, atrial fibrosis measurements, atrial connexin immunocytochemistry and myocardial angiotensin II measurements. Invasive electrophysiologic stimulation, using the same attempts in each group, induced more atrial arrhythmias in $HopX^{Tg}$ mice (48 episodes in 13 of 15 $HopX^{Tg}$ mice versus 5 episodes in 2 of 15 TSA-HopX mice, $P<0.001$; versus 9 episodes in 2 of 15 WT mice, $P<0.001$; versus no episodes in any TSA-WT mice, $P<0.001$). TSA reduced atrial arrhythmia duration in $HopX^{Tg}$ mice (1307±289 ms versus 148±110 ms, $P<0.01$) and atrial fibrosis (8.1±1.5% versus 3.9±0.4%, $P<0.001$). Atrial connexin40 was lower in $HopX^{Tg}$ compared to WT mice, and TSA normalized the expression and size distribution of connexin40 gap junctions. Myocardial angiotensin II levels were similar between WT and $HopX^{Tg}$ mice (76.3±26.0 versus 69.7±16.6 pg/mg protein, P=NS). Therefore, it appears HDAC-inhibition reverses atrial fibrosis, connexin40 remodeling and atrial arrhythmia vulnerability independent of angiotensin II in cardiac hypertrophy.

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1. Introduction

Atrial fibrillation (AF) is the most common clinical cardiac arrhythmia encountered and is highly prevalent in heart failure. In particular, left ventricular hypertrophy and diastolic dysfunction are independent risk factors for the development of AF [1,2]. Antiarrhythmic drug therapy has a relatively low efficacy for restoring and maintaining sinus rhythm. However, the discovery pulmonary vein triggers initiate AF has led to catheter-based techniques as a reliable therapy for this disease. Pulmonary vein isolation offers benefits over antiarrhythmic drugs, and is effective in patients with normal hearts, but present techniques are less effective in the setting of structural heart disease such as left ventricular hypertrophy [3].

Atrial electrical remodeling contributes to the perpetuation of AF, although it may also be a consequence of the arrhythmia. In this regard, evidence suggests atrial structural changes alone are sufficient to promote AF [4]. Angiotensin-inhibition affects myocardial remodeling and fibrosis through its action upon multiple pathways. These include transforming growth factor beta-1 (TGF-β1) signaling, and mitogen-activated protein kinases (MAPK). Experimental and clinical evidence support the ability of angiotensin-inhibition to reduce AF [5], however angiotensin-independent pathways also contribute to atrial structural remodeling and AF [6]. Therefore, elucidation of angiotensin-independent pathways that promote atrial structural remodeling may lead to novel therapies for AF in the setting of left ventricular hypertrophy.

In this report we present evidence histone-deacetylase inhibition (HDACi) reverses atrial fibrosis and arrhythmic inducibility in HopX transgenic mice with left ventricular hypertrophy. Cardiac hypertrophy induced by HopX over-expression is associated with atrial fibrosis and increased AF inducibility, but does not affect myocardial angiotensin II levels. Therefore, in this particular model HDACi reduces atrial arrhythmogenesis through favorable effects upon atrial structural remodeling independent of angiotensin.

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2. Materials and methods

Key methodological components used are described below in abbreviated form. A full description of all methods is available in the online Data Supplement.

2.1. Animals

Creation of HopX transgenic mice ($HopX^{Tg}$) has been previously described [7]. Fourteen to eighteen week-old $HopX^{Tg}$ (TSA-HopX) and wild-type (TSA-WT) mice were administered 0.6 mg/kg/day Trichostatin A (TSA, Sigma-Aldrich) by intraperitoneal injection for 14 days; and compared to age-matched $HopX^{Tg}$ mice injected with saline for the same duration or wild-type littermates given no treatment. All protocols conformed to the guidelines established by the Association for the Assessment and Accreditation of Laboratory Animal Care and were approved by the University of Pennsylvania Animal Care and Use Committees. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. In vivo electrophysiology

Four groups of animals, $HopX^{Tg}$, WT, TSA-WT (n=15) and TSA-HopX mice (n=10) were anesthetized with pentobarbital (33 mg/kg IP) and multi-lead ECGs obtained. An octapolar 1.7-French electrode catheter (CIBer mouse-EP; NuMED) was placed in the right atrium and ventricle under electrogram guidance through a jugular vein cutdown. A programmed digital stimulator (DTU-215, Fischer Scientific) delivered electrical impulses at approximately twice diastolic threshold, while surface ECG and intracardiac electrograms were displayed on a multichannel oscilloscope recorder (Bard Electrophysiology, Inc.) and analyzed offline. We defined an arrhythmic episode as induction of three or more consecutive ectopic beats following the last extrastimuli.

2.3. Invasive hemodynamics

Invasive hemodynamic recordings were obtained from the four groups of mice (n=7 in each group). Anesthesia was induced by ventilation with isoflurane. A microtip pressure–volume catheter (SPR-839; Millar Instruments) was advanced into the left ventricular via the right carotid artery to measure intracardiac pressures. Traces were digitized at 2-kHz using a PowerLab/16 SP A/D converter (ADInstruments Ltd.) and analyzed offline.

2.4. Echocardiography

Mice from the four different groups (n=7 in each group) were anesthetized using an integrated isoflurane-based system. Two-dimensional images were obtained at 180 frames/second using a 30-MHz probe (RMV 707B, Visual Sonics) in the parasternal long- and short-axis views to obtain left atrial dimensions and guide M-mode analysis at the mid-ventricular level. Pulsed doppler recordings were obtained in the apical four-chamber view from the mitral valve and pulmonary veins. LV fractional shortening, ejection fraction and wall dimensions were computed from M-mode measurements.

2.5. Histological analysis of fibrosis

Animals were euthanized using pentobarbital overdose and whole hearts immersed in 2% neutral buffered formalin for 24 h (n=3 in each group). Fixed hearts were embedded in paraffin, sectioned (5 μm) and stained with Masson's trichrome. For each section, non-overlapping photomicrographs (400×) were taken from the entire left atrium up to, but not including, the mitral annulus. Sections were analyzed using the ImageJ software (NIH) to compute fractional area of fibrosis (blue regions) as a percentage of total myocardial area.

2.6. Tissue angiotensin II assay

Whole hearts were isolated from $HopX^{Tg}$ and TSA-HopX mice (n=3 in each group) homogenized in acetic acid and cleared by centrifugation. Supernatants were purified on a C18 Sep-Pak column (Waters Associates) and eluted with acetonitrile and trifluoroacetic acid. Angiotensin II concentration was determined from paired samples, with equal amounts of protein, by ELISA using an anti–angiotensin II antibody (Peninsula ELISA) and biotinylated angiotensin II as a tracer.

2.7. Adult atrial myocyte isolation

Mice were heparizined (100 units IP), anesthetized with pentobarbital (50 mg/kg) and hearts excised through a sternotomy (n=3 in each group). Hearts were mounted on a Langendorf apparatus and perfused with Ca^{2+}-free Tyrode's solution with collagenase B and D plus protease. When the hearts became pale and flaccid they were removed from the Langendorf apparatus, the atria dissected away and sections of atrial tissue gently triturated with a Pasteur pipette to dissociate individual myocytes.

2.8. Quantitative confocal immunodetection of atrial connexins

For immunohistochemical analysis of atrial sections, hearts were quickly excised from euthanized, heparized mice (n=3 in each group). They were rinsed of blood and then either snap-frozen in liquid nitrogen or fixed in 4% formalin in PBS, embedded in paraffin and sectioned. Isolated adult atrial myocytes were fixed in 2% paraformaldehyde for 15 min. Atrial sections and myocytes were heated in 1× Antigen Unmasking Solution (Vector Laboratories) in a microwave oven to expose the epitope, and then blocked with 5% skim milk in PBS for 30 min. Frozen sections were incubated with rabbit polyclonal anti-connexin40 (1:20, Chemicon), fixed sections with rabbit polyclonal anti-connexin43 (1:20, Zymed) and fixed myocytes were exposed to both antibodies. Sections and myocytes were rinsed in PBS, incubated with the appropriate secondary antibodies and mounted. The area of immunoreactive signal, in discrete spots from atrial sections or isolated myocytes, was quantified using single optical slices (<1 μm) on the Leica TCS SP2 laser confocal system. The area of connexin immunoreactive spots and total myocardial area were quantified using the Metamorph software package (v7.2, Molecular Devices).

2.9. Western blot analysis

Protein lysates (20 μg per lane) were separated using 4–12% PAGE-SDS electrophoresis and transferred onto PVDF membranes. The membranes were blocked and blotted with primary antibodies at 1:1000 against TGF-β, phospho-ERK1/2, phospho-JNK1/2, phospho-p38-MAPK, IL-1β, connexin40, connexin43, acetylated histone H3 and GAPDH. Blot densitometry was determined using NIH ImageJ software (http://rsb.info.nih.gov), and protein band density was expressed relative to GAPDH.

2.10. Quantitative RT-PCR

Protocols for quantitative RT-PCR were performed using SYBR Green according to the manufacturer's protocol. Briefly, total RNA was isolated from whole atria of $HopX^{Tg}$ (n=4), WT (n=5) and TSA-HopX (n=5) mice. Reactions were performed in triplicate with and without RT as controls. Cycle threshold values were converted to relative gene expression levels normalized to GAPDH using the $2^{-\Delta\Delta C(t)}$ method.

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