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Original article

Remodeling of the intercalated disc related to aging in the mouse heart

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ABSTRACT

Background: Aging is related to declined cardiac hemodynamic function. As pumping performance may be significantly related to slowed ventricular depolarization and non-synchronous contraction, we hypothesized that aging may cause dysfunction of intercalated disc (ID), which is the structure responsible for intercellular electrical communication between cardiomyocytes.
Methods: Male C57BL/6J mice were used for the study at two ages: 4 and 24 months. Electrocardiographic recording was made to analyze the time of ventricular depolarization. Then mice were killed, and the hearts were harvested for examination in transmission electron microscopy (TEM) and immunofluorescence imaging. The expression of connexin 43 (Cx43), N-cadherin, and β-catenin in the myocardium of the left ventricle was evaluated using Western blotting.
Results: In senescent mice, analysis of averaged QRS complex showed its significant prolongation. At the ultrastructural level, we found frequent disruptions of the ID (affecting 29 ± 5% of them), mainly at the site of adherens junction, with relatively preserved desmosomal intercellular connections and diminished number of gap junctions. Western blotting revealed significantly decreased abundance of Cx43 protein in aged animals, which may cause slowed impulse propagation through the gap junctions and contribute to the observed electrocardiographic alterations. The level of RNA for Cx43 is similar between young and old animals, which suggests a post-transcriptional mechanism of Cx43 protein downregulation.
Conclusions: Our study shows age-related disorganization of ID, which may be responsible for slowed conduction of the depolarization wave within the heart, and supports the hypothesis of cardiac dysfunction in senescence.

Introduction

Aging is related to morphological changes of myocardium and alteration in the cardiac performance. Left ventricular hypertrophy, fibrosis, and depressed systolic and diastolic function have been reported in senescent human and animal hearts [1]. These changes, not necessarily leading to overt heart failure, decrease the cardiac reserve and make the heart more susceptible to injury by common factors, such as pressure or volume overload or by ischemia. Age-related changes in myocardial morphology affecting hemodynamics have been described in laboratory animals [2,3]. In addition to changes in the intrinsic properties of the force development in the single cardiomyocyte, impaired cardiac hemodynamics may result at least in part from less simultaneous excitation of the heart [4]. In an exaggerated presentation, it is seen in patients with injured heart and intraventricular conduction defect, in whom significant improvement in cardiac performance after resynchronization of the left ventricular excitation with the pacemaker is frequently observed [5]. Human data provided by large-scale electrocardiographic (ECG) analysis reveal age-related prolongation of the QRS, suggesting decline in the velocity of the excitation wave spreading throughout the myocardium [6]. However, in animal models, much less is known about ventricular depolarization disturbances in senescence. Proper propagation of

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the electrical impulse throughout the myocardium is possible with preserved structure and function of the intercalated discs (ID). They connect adjacent cells with the mechanical joint, formed by adherens junction (AJ) and desmosome (Des). The first bridges actin cytoskeleton of the adjacent cells and the second, being reinforcement to the AJ, is anchored to the intermediate filaments. The third component of the junctional complex is the gap junction (GJ), which contains clusters of low-resistance ionic channels, each formed by a pair of connexin hemichannels. Each of these hemichannels is built into the sarcolemma at the GJ site and forms a stable noncovalent complex with its counterpart in the adjacent cell. In the ventricular myocardium, connexons are mainly formed by connexin 43 (Cx43). Close proximity of the neighboring plasma membranes, that depends mainly on preserved AJs and Des, is crucial for maintenance of the GJ channels [7]. Rapid ion transfer between cells permits coordinated depolarization of the cardiac cells and allows these cells to work as a syncytium and ordered excitation allows for the most efficient external work to eject the stroke volume.

The aim of the study was to assess the ultrastructure of ID in senescent mice and to relate its morphology to the functional, electrocardiographic index of the impulse conduction velocity within the ventricular myocardium.

Material and methods

Animals

Sixteen, male, 4-month-old (4 M group) and sixteen, 24-month-old (24 M group) C57BL/6J mice were used in the study. Animals were obtained from the Center of Experimental Medicine of the Medical University of Bialystok and were originally purchased from Jackson Laboratories (Bar Harbor, ME, USA). The weight of the 4 M animals was 31.54 ± 2.06 g and for 24 M it was 33.03 ± 2.76 g. Animals were kept in constant temperature of 22 ± 1 °C in 12:12 dark–light cycle with constant access to standard chow and water.

The experimental procedures were carried out according to the European Council Directive of 24 November 1986 (6/609/EEC) and were approved by the Local Animal Ethics Committee at the Medical University of Bialystok.

Electrocardiography

Ten mice from each group were placed into the anesthesia induction chamber flushed with 2% isoflurane for about 30–40 s to achieve brief anesthesia. Precise anesthetic delivery was provided by rodent Halovet Vaporizer (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Then animals were quickly placed on the ECG acquisition table. The gel pad electrodes were placed on the front limbs. The signal was acquired using the Animal BioAmp (ADInstruments, Bella Vista, New South Wales, Australia) and PowerLab (ADInstruments) analog-to-digital interface connected to the computer and recorded with LabChart 7 Pro software (ADInstruments) for the period of sedation that lasted for at least 20 s. Analysis was performed using the ECG analysis LabChart module (ADInstruments). The width of the QRS complex was measured on the QRS averaged from at least 30 evolutions using the method proposed previously [8]. Briefly, the beginning of the depolarization was the first detectable deflection of the QRS, while the end was settled at the isoelectric line just at the beginning of the second main wave which in previous papers was referred to as the J wave or Tr wave, and which was reported to evince the early repolarization [9]. The markers of the beginning and the end of the QRS complex were adjusted manually.

Histology and immunofluorescence

Mice were killed by cervical dislocation and hearts were immediately excised, rinsed with ice-cold phosphate-buffered saline (PBS), and samples were collected for histological examinations, ultrastructural examination, and molecular analyses. Left ventricular myocardium was split into samples. One of these samples was immersed in the freezing mounting medium and frozen in liquid nitrogen immediately after harvesting and another was put into the phosphate-buffered formalin and then processed into paraffin blocks. Paraffin-embedded tissues were sectioned into 4-μm thick sections and stained with picro-sirius red [10]. From each section, 5 random view areas were photographed under 20× magnification. Collagen content was estimated using an automated quantification performed with Image J software (National Health Institute, Bethesda, MD, USA). Frozen samples were sectioned into 5 μm-thick sections and used for immunolabeling. After blocking with 10% donkey serum/PBS, sections were incubated with anti-Cx43 (#3512, Cell Signaling, Danvers, MA, USA; 1:500). Beta-catenin immunolabeling was performed to delineate the ID using anti-β-catenin antibody (#sc-1496, SantaCruz Biotechnology, Santa Cruz, CA, USA; 1:200); as in cardiomyocytes, it is expressed solely at the IDs. In addition to individual staining procedures, the mixture of the above antibodies was used to co-localize both antigens in ID. After washing, the secondary antibodies (anti-rabbit IgG conjugated with biotin: #711-065-152, JacksonImmuno, West Grove, PA, USA, and anti-goat IgG conjugated with cyanine Cy3 – #705-165-147, JacksonImmuno) were then applied for 1 hour. Subsequently, slides were incubated with streptavidin-Alexa Fluor® 488 conjugate (#ab150089, Abcam, Cambridge, UK) and nuclei of cells were counterstained with Hoechst 33258 (Sigma-Aldrich, St. Louis, MO, USA; 1:5000). Finally, slides were coverslipped using Dako Fluorescence Mounting Medium (#S3023, Dako, Glostrup, Denmark) and evaluated using Olympus BX41 microscope equipped with the epifluorescence module and Olympus XC30 digital camera and the confocal imaging system FluoView FV10i (Olympus, Tokyo, Japan). Low magnification confocal images were acquired at 1.8 μm and high magnification images at minimum achievable thickness of the confocal layer −0.8 μm.

Transmission electron microscopy

Approximately, 1 mm^3 of the left ventricular myocardium was taken from 4 animals of both young and aged groups. After brief rinsing in ice-cold PBS, samples were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (CB) for 3 h at 4 °C. Then samples were washed in CB at 4 °C and postfixed in 1% osmium tetroxide in CB for 1 h at 4 °C and next dehydrated through a graded series of ethanol and embedded in Glycid ether 100 (Serva, Heidelberg, Germany). Ultrathin sections were contrasted with uranyl acetate and lead citrate and mounted on nickel grids and evaluated in a transmission electron microscope OPTON 900 PC (Zeiss, Oberkochen, Germany). Electronograms were acquired with the frame transfer CCD camera and evaluated using the ImageSP software (Zeiss, Germany). In each animal, 50–100 IDs were inspected and the width of the intercellular space was measured.

Western blot

Western blotting procedure was performed as described previously [11]. Briefly, eight samples of protein extracts from each group (50 μg of protein per lane) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes (BioRad, Hercules, CA, USA). We