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Neuropilin 1 ameliorates electrical remodeling at infarct border zones in rats after myocardial infarction

Hua-Zhi Wen*, Ping Xie, Fu Zhang, Yu Ma, Yan-Ling Li, Sheng-Kai Xu

Department of Cardiology, Gansu Provincial People's Hospital, China

A B S T R A C T

Background: Electrical remodeling at infarct border zone (IBZ) has been shown to contribute to the occurrence of ventricular arrhythmias after myocardial infarction (MI). Sema3A has been demonstrated to reduce the inducibility of ventricular arrhythmias. Neuropilin 1 (NRP1) is the receptor of Sema3A. In the present study, we investigated whether treatment with NRP1 can ameliorate electrical remodeling at IBZ after MI.

Methods and results: Wistar rats underwent sham operation (n = 20), the ligation of left coronary artery (MI group, n = 30), MI with control adenovirus (Ad group, n = 30), and MI with NRP1 adenovirus (NRP1 group, n = 30). Eight weeks after treatment, electrophysiological properties including heart rate variability (HRV), monophasic action potential duration (MAPD), effective refractory period (ERP) and the inducibility of ventricular arrhythmias and the expression of arrhythmia-related ion channel proteins including Kv4.2, Kv4.3, KChIP2 and Kir2.1 at the IBZ of the left ventricle were examined. Compared with the MI or Ad group, NRP1 significantly increased HRV and shortened MAPD and ERP (all $P < 0.05$). Inducibility of VT by electrophysiological study was significantly lower in the NRP1 group than in the MI or Ad group ($P < 0.05$). The expression levels of Kv4.2, Kv4.3, KChIP2 and Kir2.1 proteins were significantly decreased in MI group and Ad group. In contrast, the expression levels of these proteins were restored in NRP1 group, which may represent the molecular basis of the NRP1-mediated inhibition of electrical remodeling.

Conclusions: NRP1 can ameliorate electrical remodeling at IBZ after MI.

1. Introduction

Sudden cardiac death (SCD) remains a major and unresolved problem. Despite advances in management strategies and patient education in prevention of SCD, morbidity and mortality due to arrhythmias in myocardial infarction (MI) are still high. Malignant ventricular tachyarrhythmia is one of the most frequent causes of SCD in patients with myocardial ischemia or infarction (Fong et al., 2011; Kazmierczak et al., 2011). Increasing studies demonstrated that electrical remodeling at infarct border zone induced by MI played a critical role in the occurrence of ventricular arrhythmias (Huang et al., 2001; Li et al., 2004).

The semaphorins, a large family of secreted and membrane-associated molecules, are widely expressed in the embryonic nervous system and have been found to play an important role in axonal guidance, fasciculation, branching and synapse formation (Bagri et al., 2003; Goshima et al., 2000; Kolodkin, 1998). Semaphorin3A (Sema3A) is a secreted protein that regulates axon/dendrite growth and neuronal migration (Goshima et al., 2000; Kawasaki et al., 2002; Kolodkin, 1998). It initiates growth cone collapse, inhibits axonal outgrowth, and plays crucial roles in neural, cardiac and peripheral vascular patterning (Goshima et al., 2012).There is evidence that appropriate expression of Sema3A in cardiac tissue is needed for sympathetic innervation and is critical for heart rate control. When Sema3A overexpressed, the rat demonstrated sudden death and susceptibility to ventricular tachycardia, due to prolongation of the action potential duration (Ieda et al., 2007). Recent studies demonstrated that upregulating Sema3A by transfecting the Sema3A gene into the infarct border zone could reduce sympathetic hyper-reinnervation and inducible ventricular arrhythmias in post-infarcted hearts (Chen et al., 2013; Hu et al., 2016; Wen et al., 2011). Moreover, variations in the Sema3A gene were identified in unexplained cardiac arrest patients with documented ventricular fibrillation (Nakano et al., 2013). Neuropilin 1 (NRP1) is the receptor of Sema3A, mediates the effects of Sema3A. NRP1 is expressed not only in the sensory neurons of the dorsal root ganglia but also in autonomic neurons of the sympathetic ganglia, both of which derive from a common progenitor, the trunk neural crest cells of the ventral migratory pathway. Previous study showed that the NRP1 -mediated Sema3A signals regulate arrest and aggregation of sympathetic neuron precursors and sympathetic neurons themselves at defined target sites and axon fasciculation to produce the stereotyped sympathetic nerve pattern (Chen et al., 1998; Nangle and Keast, 2011; Wehner et al., 2016). The purpose of this present study was to investigate whether NRP1 could ameliorate electrical remodeling at infarct border zone in MI rats.

* Corresponding author.
E-mail address: wenhz2010@163.com (H.-Z. Wen).

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2. Materials and methods

2.1. Animal preparation

All animal experimental procedures were approved by the medical ethics committee, Gansu Provincial People's Hospital, and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th edition, 2011).

The Wistar rats were supplied by the animal experiment centre of Wuhan University, China. Male rats weighing 250 to 300 g were divided randomly into 4 groups: sham-operated rats treated with sham operation (SO group, n = 20), MI rats (MI group, n = 30), MI rats with control virus (Ad group, n = 30), and MI rats with NRP1/adenoviruses (NRP1 group, n = 30). MI was induced by left coronary artery ligation as previously described (Wen et al., 2010, 2011). A sham operation was performed to expose the rat heart without coronary artery ligation in the SO group.

2.2. Construction of adenoviral vectors

Replication-defective recombinant adenoviruses (Ad) expressing green fluorescent protein (GFP/Ad, control virus) and NRP1 (NRP1/Ad) were constructed as described previously (Wen et al., 2011). All plaque-purified adenoviruses were examined for replication-competent adenoviruses by PCR and were amplified and purified by double cesium chloride gradient ultracentrifugation. The absolute concentration of viral particles was determined by optical absorbency, and the concentration of infectious particles (plaque-forming units) was quantified by viral hexon protein expression in infected human embryonic kidney 293 (HEK293) cells using the Adeno-X Rapid Titer kit (BD Biosciences, Franklin Lakes, NJ). Expression of each protein by virus-infected U373 cells was confirmed by Western blot 72 h after transfection as described previously (Pellet-Many et al., 2011; Pellet-Many et al., 2015). The quality of these viruses was determined by examining the ratio of infectious to total (live and dead) number of virus, which was > 1:50 plaque-forming unit (pfu) to total virus particles.

2.3. Adenovirus injections

GFP/Ad and NRP1/Ad were injected into the infarct border zone in Ad and NRP1 groups, respectively, after the ligation of coronary artery for 30 min. The infarct border zone was defined as the zone within 3 mm of the marginal zone between the infarct zone and the non-infarct zone. Each virus administration consists of eight injections (0.5 μl/each, GFP or NRP1/Ad) along the infarct border zone. All injections were performed using a beveled glass micropipette pulled to an external diameter of 30 to 50 μm. After operation was accomplished, all animals were fed with standard diet for 8 weeks.

2.4. Hemodynamic measurement

Eight week after MI, a catheter was inserted into the right common carotid artery to record left ventricular systolic pressure (LVSP), left ventricular end- diastolic pressure (LVEDP) and the maximum rise and fall (± dp/dt) rate of left ventricular pressure. Heart rate (HR) was monitored with a computer-based electrophysiology system (LEAD2000B; Jinjiang Ltd., Chengdu, China).

2.5. Analysis of heart rate variability

Surface ECGs were continuously recorded with a computer-assisted PC-Lab system (Weixinsida Technology CO., Ltd., Beijing, China) for 2 h for each animal with a sample rate of 1000 Hz. Five-minute segments at 30-minute intervals were used for analysis of heart rate variability (HRV). To avoid the effect of circadian oscillation of heart rate on the HRV evaluation, we restricted the ECG recording time to 9:00–11:00 am for all subjected animals. R waves were detected off-line with wavelet transform algorithm and then by manual artifact removing. Linear parameters of HRV, including mean R–R intervals, standard deviation of the normal-to-normal R–R intervals (SDNN) and root mean square of successive difference (RMSSD) of R–R intervals, and non-linear parameters, including approximate entropy (ApEn) and Poincaré plot, were analyzed as previously described (Ren et al., 2008; Wen et al., 2011).

2.6. Electrophysiological studies

Eight weeks after surgery, all surviving rats underwent re-thoracotomy to expose the hearts. Respiration was maintained by the same volume-controlled rodent respirator. A pair of platinum needle electrodes (0.1 mm) were inserted into the peri-infarct zone in the MI group, control group and NRP1 group or the corresponding zone in the SO group. The peri-infarct zone was defined as the zone within 3 mm of the marginal zone between the infarct zone and the non-infarct zone. All signals were recorded with a polygraph (LEAD2000B, Jinjiang Ltd., Chendu, China) and were filtered between 50 Hz and 300 Hz. The band pass filter was set at 50–300 Hz for standard cardiac electrogram recording and at open 300 Hz for recording of the monophasic action potential (MAP). The MAP duration (MAPD) was determined as the interval between the onset of the MAP trace and the 90% repolarization time ($MAPD_{90}$). To evaluate the ventricular effective refractory period (ERP), we used square wave impulses of 2 ms duration at twice the diastolic threshold, which were generated by a programmable cardiac stimulator (DF-5A, Dongfang Ltd., Suzhou, China) and following a train of 8 regular stimuli (S1) with a cycle lengths of 120 ms, an early extrastimulus (S2) was introduced and subsequently introduced progressively in 5 ms steps until it failed to trigger an action potential. The ERP was defined as the longest S1S2 interval at which S2 failed to produce a propagated ventricular response (Wakisaka et al., 2004).

The end point of electrophysiological studies was induction of a ventricular tachyarrhythmia consisting of at least 6 consecutive non-driven ventricular extrastimulus beats. Distinction was not made between ventricular tachycardia and ventricular fibrillation. A ventricular tachyarrhythmia was considered nonsustained when it lasted ≤15 beats and sustained when it lasted > 15 beats before terminating spontaneously or by overdrive pacing. An arrhythmia scoring system was used as described (Curtis and Walker, 1988).

2.7. Infarct size measurement

The left ventricle was cut into four to six slices parallel to the base, and immersed into 1% triphenyltetrazolium chloride (TTC) solution for 1 h. The surviving myocardium was stained brick red. The percentage of myocardial infarction was determined by the ratio between the white (infarcted) area and the total area of the left ventricle. Area measurements were performed by Image-Pro Plus 4.0 (Media Cybernetics, Silver Spring, MD, USA).

2.8. Western blot analysis

The peri-infarct zone of left ventricle samples were homogenized in lysis buffer containing proteinase and phosphatase inhibitors. An equal amount of protein (40 μg) was loaded into a 10% SDS-PAGE. Unspecific bindings were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TTBS) at room temperature for 2 h and then were incubated overnight at 4 °C in TTBS containing 0.5% non-fat dry milk and rabbit anti-rat Kv4.2, anti-rat Kv4.3, anti-rat Kir2.1 (Sigma, dilution 1:1000) and mouse anti-KChIP2 (Sigma, dilution 1:500) primary antibodies, respectively. The expression of proteins was normalized to glyceraldehydes-3-phosphate dehydrogenase (GAPDH) expression.