Spatial distribution of nerve sprouting after myocardial infarction in mice

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BACKGROUND Myocardial infarction (MI) elicits nerve sprouting.

OBJECTIVES The purpose of this study was to determine the spatial distribution of nerve sprouting and neurotrophic gene expression after MI.

METHODS We created MI in mice by coronary artery ligation. The hearts were removed 3 hours to 2 months after MI and examined for nerve fiber density and neurotrophic factor gene expression using Affymetrix microarray and mRNA analyses.

RESULTS The density of nerve fibers immunopositive for growth-associated protein (GAP)-43 was the highest 3 hours after MI both in the peri-infarct area and in the area remote to infarct, resulting in sympathetic (but not parasympathetic) hyperinnervation in the ventricles. The GAP-43–positive nerve fiber density of myocardium was greater in the outer transverse loop than in the inner vertical loop. The differences between these two myocardial loops peaked within 3 hours after MI and persisted for 2 months afterward. Gene expression of nerve growth factor, insulin-like growth factor, leukemia inhibitory factor, transforming growth factor-β_3, and interleukin-1α was increased up to 2 months after MI compared with normal control. Expression of these growth factors was more pronounced and persistent in the peri-infarct area than in the remote area.

CONCLUSION MI induces sympathetic nerve sprouting in both peri-infarct and remote areas, more in the outer transverse loop. Selective up-regulation of nerve growth factor, insulin-like growth factor, leukemia inhibitory factor, transforming growth factor-β_3, and interleukin-1α occurred in the peri-infarct area and, to a lesser extent, in the remote area.

KEYWORDS Myocardial infarction; Helical heart; Neurotrophic factors; Nerve sprouting; Sympathetic nerve; Cholinergic nerve; Neural regeneration; Neural remodeling; DNA microarray

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Introduction

Injury of many types, including division, crushing, and disruption of adequate blood supply, causes Wallerian degeneration of peripheral nerves. This may be followed by neurilemma cell proliferation and axonal regeneration.[1] Like many organs, the vertebrate heart is well innervated by autonomic nerves.[2] Myocardial infarction (MI) causes cardiac nerve injury and denervation.[3] This may be followed by nerve sprouting in both animals and humans.[4–7] The nerve fiber regeneration process is triggered by up-regulation of nerve growth factor (NGF) or other neurotrophic factor genes in the non-neuronal cells around the site of injury.[8] We previously reported an increase of NGF protein and mRNA expression 3 days to 1 month after MI in a canine model.[9] Up-regulation of NGF after MI was more prominent in the area near the infarct than in the area remote to the infarct. Whether or not other neurotrophic factors are up-regulated in the myocardium after MI is unclear. Based on our previous observations,[7,9,10] we hypothesize that MI induces up-regulation of various neurotrophic factors and elicits nerve sprouting activity more persistent around the lesion.

In this study, we used the small size of the murine heart to our advantage to quantify the anatomic distribution of nerve sprouting 3 hours to 2 months after MI. We also performed DNA microarray on a subset of mouse hearts to study gene expression of neurotrophic factors after MI. The main goal was to study the spatial relationship between nerve sprouting activity and MI and between nerve sprouting activity and gene expression of growth factors. We measured nerve sprouting activity by

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quantifying the immunoreactivity of growth-associated protein-43 (GAP-43), a protein up-regulated in axons undergoing nerve sprouting. We also attempted to differentiate sympathetic from parasympathetic nerve fiber by immunostaining tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT).

The architectural arrangement of ventricular muscle mass resembles that of a Gordian knot,[11] with an outer (transverse) loop and an inner (vertical) loop forming a helical structure. Both the sequence and the force of the contraction in this helical structure may be determined by the cardiac innervation. Although this new concept of ventricular architectural arrangement is considered important in determining ventricular function,[12] no data on the distribution of cardiac nerves in the helical structure are available. Therefore, we also took the advantage of small murine hearts to study nerve sprouting activity in the helical structure after MI.

Materials and methods

The study protocol was approved by the Institutional Animal Care and Use Committee and followed the guidelines of the American Heart Association.

Surgery for creation of MI in mice

FVB mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). The mice were anesthetized intraperitoneally with injection of ketamine (40–50 mg/kg) and xylazine (40–50 mg/kg). Hair on the chest and overlying the trachea was clipped and the skin prepared with povidone-iodine (Betadine) and alcohol. Using sterile techniques, the skin overlying the trachea was opened. The mice were intubated under direct visualization by inserting a size 60 polyethylene tube from the mouth into the trachea. The tube was connected to a volume-cycled rodent respirator (Harvard Apparatus, South Natick, MA, USA) that provided positive-pressure ventilation at 200 to 300 μL/cycle and a respiratory rate of 120 cycles/min. Following confirmation of adequate ventilation, the thoracic cavity was opened, and ligation of the left coronary artery was performed 3 to 4 mm from the tip of the left auricle using 7-0 silk suture. ECG was recorded to document ST-segment elevation after ligation (Figure 1A). A group of mice was sacrificed 3 hours after MI. In the remaining mice, the chest was closed with continuous 6-0 polypropylene (Prolene) suture, and pleural air was evacuated with tubing connected to a syringe before all skin wounds were closed using 4-0 polyester sutures. Animals were extubated once they were breathing spontaneously. The mice were kept warm by a heat lamp or heating pad and watched until they fully recovered from anesthesia and surgery. Buprenorphine (0.05–0.1 mg/kg) was administered subcutaneously for postoperative analgesia. In this study, eight of 42 mice died of surgical complications. The remaining 34 MI mice were used for data analyses.

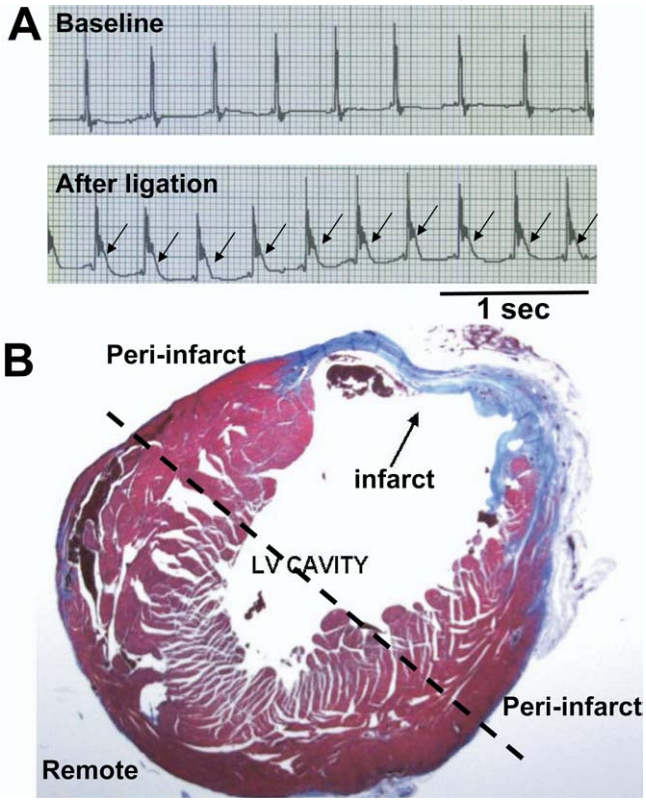

Figure 1 Mouse myocardial infarction created by left coronary artery ligation. **A:** Surface ECG at baseline and after left coronary ligation. ST-segment elevation occurred after coronary artery ligation *(arrows)*, indicating myocardial ischemia. Note heart rate acceleration (as indicated by closer approximation of QRS complexes) after coronary artery ligation. **B:** Cross-section of the left ventricle (trichrome stain). The fibrous tissues stained *blue* and the myocardium stained *red*. *Dashed line* drawn halfway through the tissue indicates separation of the peri-infarct region from the remote region.

Heart tissue preparation

Mice were anesthetized, the chests opened, and the hearts quickly excised. For immunohistochemistry, the whole heart was fixed in 4% formalin for 45 minutes to 1 hour, followed by storage in 70% alcohol.[10] The hearts used for microarray analyses were dissected into three parts: infarct, peri-infarct, and remote (Figure 1B). First, the infarct area, which could be identified visually, was cut out. Then the rest of the heart was cut into two equal parts: the half that was close to the infarct was the peri-infarct area, and the other half was the remote tissue. Both the peri-infarct and remote tissues were frozen separately in liquid nitrogen and stored in −80°C for later processing (see later).

DNA microarray

Affymetrix (Santa Clara, California) GeneChips (Mouse Genome U74Av2) were used for mRNA expression profiling (transcriptomics). Experimental procedures for gene chips were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual. RNA was isolated from tissue using the Qiagen RNeasy mini-