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The lack of cardiotrophin-1 alters expression of interleukin-6 and leukemia inhibitory factor mRNA but does not impair cardiac injury response

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Abstract

Cardiotrophin-1 (CT-1) was identified as a growth factor for cardiac myocytes and CT-1 protects myocytes from cell death. Adult CT-1$^{-/-}$ mice exhibit neural deficits including the loss of preganglionic sympathetic neurons, but their autonomic and cardiac parameters have not been examined. We used these mice to determine if the absence of CT-1 or loss of preganglionic sympathetic input altered heart rate, left ventricular pressure, cardiac contractility (dP/dt), or cell death following ischemia-reperfusion. Basal heart rate was increased in CT-1$^{-/-}$ mice, and this difference was abolished by ganglionic block. Left ventricular pressure and dP/dt were unchanged. Dobutamine stimulated similar increases in heart rate and dP/dt in both genotypes, but ventricular pressure was significantly lower in CT-1 nulls. Cardiac expression of interleukin-6 (IL-6) mRNA was increased significantly in CT-1 null mice, while leukemia inhibitory factor (LIF) mRNA was unchanged. Infarct size normalized to area at risk was no different in CT-1$^{-/-}$ mice ($33.8 \pm 1.0\%$ vs. $37.7 \pm 3.2\%$ WT) 24 h after ischemia-reperfusion. Induction of IL-6 mRNA after infarct was significantly abrogated in CT-1 null mice compared to wild-type mice, but LIF mRNA-induction remained significant in CT-1 null mice and might contribute to cardiac protection in the absence of CT-1.

Keywords: gp130; Cardiac protection; Leukemia inhibitory factor; Cardiotrophin-1; Interleukin-6

1. Introduction

Cardiotrophin-1 (CT-1) is a cytokine originally identified for its ability to stimulate cardiac myocyte hypertrophy *in vitro* [1,2]. CT-1 is part of a larger family of cytokines that includes interleukin-6 (IL-6), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin-M (OSM), interleukin-11 (IL-11), cardiotrophin-like cytokine (CLC), and neuropoietin/cardiotrophin-2 (NP/CT-2). All of these cytokines share the common signaling receptor gp130. IL-6 and IL-11 use a gp130 homodimer, while the other family members activate a heterodimer composed of gp130 and the LIF receptor (LIFR) (reviewed by [3–5]).

Several lines of evidence suggest that CT-1 and related cytokines play a critical role in cardiac development and injury response. CT-1 is expressed in the developing heart tube and supports the survival of cardiac myocytes [6–9]. Mice lacking gp130 die during embryogenesis and exhibit severe ventricular hypoplasia [10], while ventricular

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wall-thickness is diminished in mice lacking post-natal expression of gp130 [11]. The absence of CT-1 during development does not generate the lethal cardiac malformations seen in mice lacking gp130 [12], but heart size and cardiac function have not been examined in these mice. Exogenous CT-1 prevents cell death during and after cardiac ischemia-reperfusion, resulting in decreased infarct size [9,13,14], while endogenous CT-1 and the gp130 receptor are elevated significantly following myocardial infarction [15,16]. These data suggest that endogenous CT-1 plays an important cardio-protective role following ischemia-reperfusion, but the role of endogenous CT-1 in cardiac protection has not been tested directly.

Although mice lacking CT-1 do not develop severe cardiac malformations, they exhibit a significant loss of at least two classes of neurons. The absence of CT-1 results in the loss of a subset of motoneurons [12,17] and in a decreased number of preganglionic sympathetic neurons [18]. Other related cytokines are involved in supporting motoneuron survival and function [17], but CT-1 appears to be the only cytokine required for the survival and maintenance of preganglionic sympathetic neurons [18]. Postganglionic sympathetic neurons projecting to the heart increase heart rate, ventricular pressure, and contractility through activation of cardiac β receptors. Thus, cardiac function in CT-1 null mice could be altered by direct effects on the heart or by the loss of sympathetic transmission.

It is not known if the absence of CT-1, or the subsequent loss of preganglionic sympathetic neurons, alters cardiac function or injury response in adult mice. The aim of this study was to determine if the lack of CT-1 altered cardiac function, cardiac autonomic control, or infarct size after acute myocardial infarction, and to investigate potential compensation by related cytokines.

2. Materials and methods

2.1. Animals

CT-1$^{-/-}$ mice are fertile [12], and were maintained as homozygous nulls, with C57Bl/6J used as wild-type controls.

2.2. Heart weight

Hearts were excised and trimmed of the great vessels and blood was rinsed out of the chambers. The whole heart was weighed and then the atria were removed and the ventricles weighed. Heart weights were compared to body weight of the animals and tibia length. Tibias were cleaned of muscle and connective tissue and measured with calipers.

2.3. Real-time PCR

Hearts were harvested 24 h after ischemia-reperfusion and stored immediately in RNAlater. RNA was isolated from the left ventricles using the Qaigen RNAeasy mini kit. Total RNA was quantified by OD260, and 200 ng of total RNA was treated with DNase and reverse-transcribed. Each reverse transcription reaction was tested by regular PCR to confirm reverse transcription (RT), and an RNA-alone control was included for each sample to test for genomic DNA contamination. Real-time PCR was performed with the ABI TaqMan Fast Universal PCR Master mix in the ABI 7500, using ABI pre-validated TaqMan gene expression assays for mouse IL-6, LIF, neuropoeitin/cardiotrophin-2, and actin as an internal control. For the PCR amplification, 2 μl of RT reactions were used in a total volume of 20 μl, and each sample was assayed in duplicate. Cytokine mRNAs were normalized to actin mRNA in the same sample. Post-infarct cytokine/actin mRNA ratios were compared to unoperated controls of the same genotype to determine the fold-increase following ischemia-reperfusion. Control and post-infarct samples of both genotypes were assayed together. Ischemia-reperfusion did not regulate the expression of actin mRNA (data not shown).

2.4. In situ mouse model of myocardial ischemia-reperfusion

Adult mice were placed in an induction chamber and anesthetized with 4% isoflurane. Once an animal was unconscious it was given pentobarbital 30 mg/kg body weight IP. Animals were placed on a heating pad, the fur on the chest and ventral neck was removed using Nair hair remover, and the skin was wiped with saline and betadine. Mice were intubated, mechanically ventilated, and maintained with 1–2% isoflurane mixed with 100% oxygen (approximately 0.2 l/min). End-tidal CO_2 was continuously monitored to verify adequate minute ventilation. Core body temperature was monitored by a rectal probe and maintained at ~37 °C, and a 5-lead ECG was monitored throughout the surgery and experimental protocol using a PowerLab data acquisition system (ADInstruments Inc., Colorado Springs, CO) on a Macintosh ibook G4.

The mouse was turned to a right lateral decubitus position and a thoracotomy performed in the 2nd or 3rd intercostal space with the aid of a dissecting microscope. A ligature (8-0 nylon mono-filament or equivalent on a taper needle) was placed around a proximal segment of the left anterior descending coronary artery and the ends of the suture were passed through a tube (PE10) with a blunted end to prevent tissue damage [19]. The ligature was tightened to induce regional myocardial ischemia, which was confirmed by ECG changes, regional cyanosis, and wall motion abnormalities. After 45 min the coronary ligature was released, and reperfusion confirmed by visible epicardial hyperemia. The ligature was left in place for re-occlusion to delineate risk size. As soon as reperfusion was verified the chest was closed in layers. A small catheter was left in the thorax for 10–20 min to evacuate air and fluids. The mice were then removed from the ventilator and repositioned every 30 min until able to maintain sternal recumbency (usually less than 15 min). Mice were then returned