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Original article

# Paraoxonase 2 prevents the development of heart failure

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ABSTRACT

*Background:* Mitochondrial oxidation is a major source of reactive oxygen species (ROS) and mitochondrial dysfunction plays a central role in development of heart failure (HF). Paraoxonase 2 deficient (PON2-def) mitochondria are impaired in function. In this study, we tested whether PON2-def aggravates HF progression.

*Methods and results:* Using qPCR, immunoblotting and lactonase activity assay, we demonstrate that PON2 activity was significantly decreased in failing hearts despite increased PON2 expression. To determine the cardiac-specific function of PON2, we performed heart transplantations in which PON2-def and wild type (WT) donor hearts were implanted into WT recipient mice. Beating scores of the donor hearts, assessed at 4 weeks post-transplantation, were significantly decreased in PON2-def hearts when compared to WT donor hearts. By using a transverse aortic constriction (TAC) model, we found PON2 deficiency significantly exacerbated left ventricular remodeling and cardiac fibrosis post-TAC. We further demonstrated PON2 deficiency significantly enhanced ROS generation in heart tissues post-TAC. ROS generation was measured through dihydroethidium (DHE) using high-pressure liquid chromatography (HPLC) with a fluorescent detector. By using neonatal cardiomyocytes treated with $CoCl_2$ to mimic hypoxia, we found PON2 deficiency dramatically increased ROS generation in the cardiomyocytes upon $CoCl_2$ treatment. In response to a short $CoCl_2$ exposure, cell viability and succinate dehydrogenase (SDH) activity assessed by MTT assay were significantly diminished in PON2-def cardiomyocytes compared to those in WT cardiomyocytes. PON2-def cardiomyocytes also had lower baseline SDH activity. By using adult mouse cardiomyocytes and mitochondrial ToxGlo assay, we found impaired cellular ATP generation in PON2-def cells compared to that in WT cells, suggesting that PON2 is necessary for proper mitochondrial function.

*Conclusion:* Our study suggests a cardioprotective role for PON2 in both experimental and human heart failure, which may be associated with the ability of PON2 to improve mitochondrial function and diminish ROS generation.

## 1. Introduction

Despite improvements in therapeutic regimens for patients with heart failure (HF), the mortality of HF remains as high as 50% within 5 years of diagnosis [1]. Patients who develop HF following cardiac insults often experience progressive, adverse cardiac remodeling and fibrosis attributed to excessive inflammation and oxidative/nitrative stress. Although various studies suggest that phase II antioxidative

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enzymes may be potential therapeutic candidates against cardiac remodeling [2], clinical trials of antioxidative approaches to prevent cardiovascular morbidity and mortality have not yet been successful [3].

Paraoxonases (PON) constitute a family of calcium-dependent esterases with three isoforms: PON1, PON2, and PON3. While all three isoforms exhibit arylesterase and paraoxonase activities, the native enzymatic activity of PON is considered lactonase [4]. Studies have shown that both PON1 and PON3 are associated with high-density lipoprotein in the circulation, whereas PON2 is expressed in various major organs as a cell-associated enzyme [5,6]. These pleiotropic enzymes are highly conserved genetically across species, with diverse roles including protection against lipid peroxidation and oxidative stress, modulation against endoplasmic reticulum stress, and regulation of cell proliferation and apoptosis [7]. Our group, as well as others, has demonstrated the important anti-oxidative role of systemic PON activities in humans. Specifically, diminished paraoxonase/arylesterase activities in serum were directly associated with increased levels of oxidized lipoproteins [8], presence of subclinical myocardial necrosis as detected by high-sensitivity cardiac troponin I [9], and increased adverse cardiac events in stable cardiac patients [10]. In addition, diminished serum arylesterase activities were associated with patient history of HF [11] or chronic kidney disease [12], as well as poorer long-term survival. These findings have now been replicated in an independent outpatient HF cohort [13].

We observed that serum paraoxonase/arylesterase activity levels strongly track with genetic polymorphisms linked to PON1 genotype (especially Q192R), thereby confirming the contribution of circulating PON1 to these esterase activities [10]. While the majority of human studies have focused on circulating PON esterase activities and their impairment in disease states, few have specifically targeted PON isoforms. Additionally, direct quantification of tissue distributions and characteristics of PON isoforms in humans is rarely performed, and the roles of PON2 and PON3 in cardiovascular diseases are less understood.

In animal studies, PON2 is ubiquitously produced in all tissues, yet is not detected in HDL or LDL like PON1 and PON3 [14]. In mice, PON2 is the most abundant PON isoform in the myocardium [5], and may contribute substantially to the lactonase activity in the myocardial tissue [15]. While subcellular localization of PON2 in the heart has not been extensively characterized, cell fractionation studies have revealed a predominant PON2 association in microsomes and lysosomes of human jejunum [16], in nuclear membrane and endoplasmic reticulum of vascular cells [17], and in the mitochondria of dopaminergic areas (e.g., striatum) and astrocytes of the brain [18]. The proximity of these intracellular locations may provide unique in situ anti-oxidative effects and cellular protection.

In this study, we demonstrate that mice with genetic deficiency of PON2 have dilated cardiac remodeling, which is aggravated upon additional cardiac insults and may be reversed via systemic overexpression of PON2. We further demonstrate that the aggravated cardiac remodeling may be associated with increased ROS generation in heart tissue upon cardiac insults. Human hearts primarily express PON2 compared to the nominal expression levels of PON1 and PON3. Although PON2 expression is higher at both mRNA and protein levels in human failing hearts compared to that in non-failing hearts, PON2 lactonase activity is lower in failing hearts. PON2 could be cardioprotective; however, under oxidative/nitrative stress, PON2 may be rendered “dysfunctional” and lose its cardioprotective effects.

## 2. Methods

### 2.1. Mice

The PON2 deficient mouse strain (PON2-def) was backcrossed into the C57BL/6 (Jackson Laboratory, Bar Harbor, ME) background for more than 10 generations to produce PON2-def animals with a homozygous genomic background. This was extensively used in our previous studies [19,20]. PON2-def mice are fertile and do not differ from WT mice in gross appearance. Mice of both genders, aged four days to four months old, were used for the study. All animal procedures and manipulations were approved by the IACUC of Cleveland Clinic in accordance with the United States Public Health Service Policy on the Humane Care and Use of Animals, and the NIH Guide for the Care and Use of Laboratory Animals.

### 2.2. Human heart tissue samples

Human failing hearts were from patients who underwent heart transplantation because of end-stage heart failure at Cleveland Clinic. All human studies have been approved by the Institutional Review Board (IRB) of Cleveland Clinic. Upon arrival to the lab, heart tissues were cut into small pieces, snap frozen in liquid nitrogen and then stored in − 80 °C for later use. Left ventricles from eight randomly selected failing hearts with ischemic cardiomyopathy were used in this study. Left ventricles of eight randomly selected unmatched donor hearts were used as non-failing controls. No clinical information was collected for this study.

### 2.3. Materials

Rabbit polyclonal antibodies against human PON1, PON2 and PON3 were generated by the Hybridoma Core at Cleveland Clinic Lerner Research Institute (Cleveland, OH). Antibodies to PON2 were also purchased from Abcam (Cat.#: ab183710, Cambridge, MA) and Santa Cruz (Cat.#: sc-374158, Dallas, TX). Antibody to actin was purchased from Santa Cruz (Cat.#: sc-81178). An adeno-associated virus (AAV) construct (AAV serotype 9), with a cytomegalovirus (CMV) promoter to drive the expression of murine PON2 (AAV9-PON2) was constructed by Vector Biolabs (Malvern, PA). All other chemical reagents were purchased from Sigma (St. Louis, MO), except where specifically indicated.

### 2.4. Heart transplantation model

To study the role of PON2 in protecting the myocardium directly, we used a heart transplantation model where the donor hearts were subjected to “hot ischemia” to enhance heart damage. Heparinized donor hearts were dissected and stored in 37 °C saline for 1 h before transplanting into WT recipient mice using the procedure described by Hasegawa et al. [21]. To evaluate graft function, we scored the donor hearts at 2 min after implantation and 4 weeks post-transplantation based on the following criteria: score 0 = no contractions; score 1 = minimal visible ventricular motion; score 2 = weak or partial ventricular contractions; score 3 = homogeneous ventricular motion at low intensity; and score 4 = normal atrial and ventricular contraction intensity [22]. The scores were evaluated by the operator at 2 min after implantation, and by two researchers blinded to the surgery and group division at 4 weeks post-transplantation. The average scores of the two researchers were assigned to each mouse and used for statistical analysis.

### 2.5. Mouse transverse aortic constriction (TAC) model of heart failure

The TAC model is a previously published, reproducible model of HF predominantly due to pressure overload [23]. The sudden onset of hypertension achieved by TAC causes both an approximately 50% increase in LV mass within the first 2–4 weeks, and a well-defined HF phenotype by 12 weeks. Following intubation and mechanical ventilation of the mice, the transverse aorta was accessed through partial thoracotomy via the upper edge of the sternum. The transverse aorta was dissected free from surrounding tissues and then ligated together with a small piece of a blunted, 27-gauge needle parallel to the aorta, using a 7–0 silk thread. The 27-gauge needle was then removed to yield