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Original Article

A mouse model of endocardial fibroelastosis

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ABSTRACT

Background: Endocardial fibroelastosis (EFE) is a pathologic condition of abnormal deposition of collagen and elastin within the endocardium of the heart. It is seen in conjunction with a variety of diseases including hypoplastic left heart syndrome and viral endocarditis. While an experimental model using heterotopic heart transplant in rats has been described, we sought to fully describe a mouse model that can be used to further elucidate the potential mechanisms of and treatments for EFE.
Materials and methods: The hearts of 2-day-old C57BL/6 mice were transplanted into the abdomen of 7-week-old C57BL/6 mice. At 2 weeks, the hearts were harvested and histologic analysis was performed using hematoxylin and eosin, Masson's trichrome, Russell-Movat's pentachrome, Picrosirius red, Hart's, Verhoeff-Van Gieson, and Weigert's Resorcin-Fuchsin stains. Additionally, one heart was analyzed using transmission electron microscopy (TEM).
Results: Specimens demonstrated abnormal accumulation of both collagen and elastin within the endocardium with occasional expansion into the myocardium. Heterogeneity in extracellular matrix deposition was noted in the histologic specimens. In addition, TEM demonstrated the presence of excess collagen within the endocardium.
Conclusions: The heterotopic transplantation of an immature heart into a mouse results in changes consistent with EFE. This model is appropriate to investigate the etiology and treatment of EFE.

1. Introduction

Endocardial fibroelastosis (EFE) is a pathologic condition characterized by expansion of the endocardium by collagen and elastin (Fig. 1). On gross examination, these changes appear as a pearly or opaque white inner lining of the heart, predominately in the ventricles [1,2]. The thickening caused by the abnormal deposition of collagen and elastin within the smooth muscle layer of the endocardium is thought to limit ventricular diastolic compliance and might also restrict ventricular growth [3,4].

EFE is reported in conjunction with a broad spectrum of diseases (Table 1), including hypoplastic left heart syndrome (HLHS) and viral endocarditis [1], which have been comprehensively reviewed in the literature. Little is known about the pathogenesis of this condition, in part due to the wide variety of circumstances in which it occurs. Some authors have suggested that an inflammatory response within the heart leads to a proliferation and migration of mesenchymal cells such as fibroblasts, myofibroblasts, or smooth muscle cells that deposit extracellular matrix constituents such as collagen and elastin within the endocardium [1,4,5].

While EFE has been described in a variety of animals such as horses, dogs, cats, ruminants, and chickens [6–10], the spontaneity with which these changes occur makes these species suboptimal for rigorous investigation. A rat model of EFE involving the heterotopic transplant of a 2-day-old donor heart into the abdomen of a syngeneic recipient rat has previously been described. Using the concept that low flow induces endocardial remodeling, the authors demonstrated that unloaded heterotopic transplant recapitulates some of the changes seen in EFE [11]. The aim of the current study was to apply these concepts to a mouse model, which is better suited to elucidate the cellular and molecular mechanisms behind this complex pathology.

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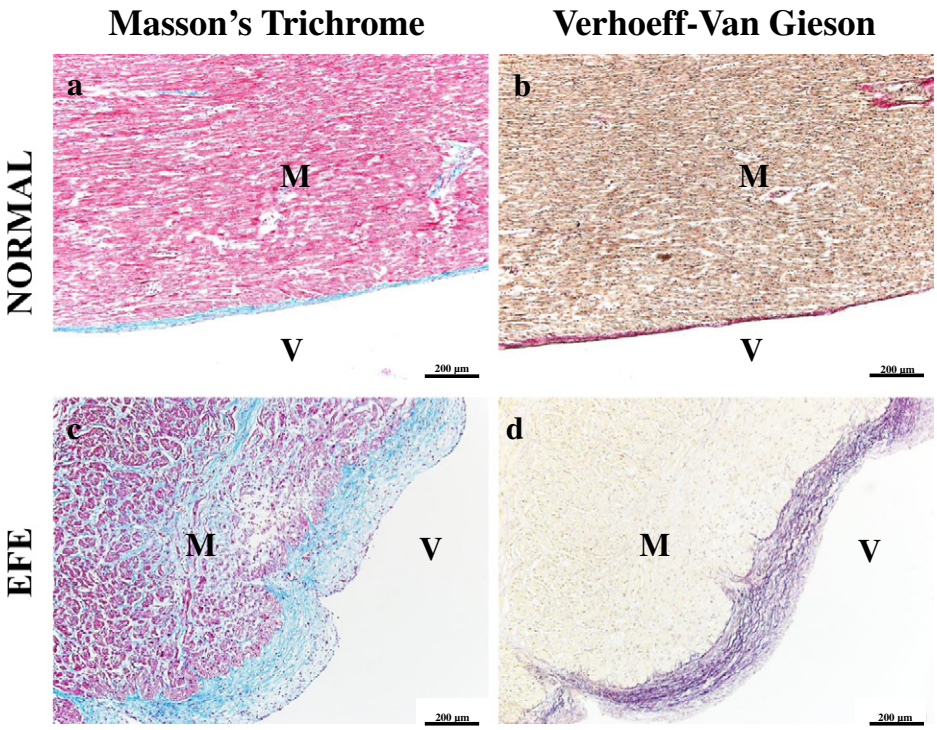

Fig. 1. EFE in an infant heart. Comparison of normal human endocardium **(a, b)** and endocardium with EFE **(c, d)**. Photomicrographs demonstrate expansion of the endocardium with collagen and elastin **(c, d)**. Masson's trichrome **(a, c)**; collagen (blue), myofibers (red); VVG stain **(b, d)**: elastin (fibrillar basophilic stain). V: ventricle; M: myocardium.

2. Materials and methods

2.1. Animal care/ethics statement

The Institutional Animal Care and Use Committee of the Research Institute at Nationwide Children's Hospital (Columbus, OH) approved and monitored the protocol for the present study. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (2011), from the Public Health Service, National Institutes of Health (Bethesda, MD).

Table 1
Diseases and other stressors associated with EFE

- **Cardiomyopathies**
  - *Dilated*
    - Postmyocarditis
    - Genetic
      - Known genes
        - Muscle LIM protein
        - C-linked fetal cardiomyopathy
        - Beta cardiac myosin heavy chain
      - Familial but idiopathic
        - Autosomal recessive
        - Autosomal dominant
        - X-linked recessive
  - *Hypertrophic*
    - Noonan syndrome (left atrium)
  - *Restrictive*
    - Postmyocarditis
    - Idiopathic
  - *Noncompaction*
    - Barth syndrome
- **Congenital malformation**
  - *Aortic stenosis*
  - *Coarctation of the aorta*
  - *Anomalous coronary arteries*
  - *HLHS*
  - *Intracranial arteriovenous fistula*
- **Immunologic disease**
  - *Transplacental maternal antibodies*
  - *Rhesus incompatibility*
- **Myocarditis**
  - *Viral*
    - Mumps
    - Coxsackie
    - Adenovirus
  - *Bacterial*
    - Lactobacillus
- **Lysosomal storage diseases**
  - *Glycogen*
    - Type II Pompe
  - *Mucopolysaccharides*
    - Hurler's
    - Maroteaux–Lamy
  - *Sphingolipids*
    - Niemann–Pick's
  - *Gangliosides*
    - Infantile Sandhoff's
- **Other metabolic**
  - *Systemic carnitine deficiency*
- **Physical injury**
  - *Postelectric shock*
- **Vascular**
  - *Myocardial infarction*
  - *Twin–twin transfusion*
  - *Lymphatic obstruction*

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2.2. Donor heart harvest

Two-day-old C57BL/6 pups (Jackson Laboratories, Bar Harbor, ME) ($n=18$) were euthanized by intraperitoneal injection of ketamine (300 mg/kg; Hospira Inc., Lake Forest, IL) and xylazine (30 mg/kg; Akorn Inc., Decatur, IL), followed by induced pneumothorax. The vena cava (left and right cranial, caudal), pulmonary artery, and aorta were identified, isolated, ligated, and transected. The heart was then placed in phosphate-buffered saline (PBS) (Gibco R, Life Technologies) on ice until the time of implantation (<10 min).

2.3. Unloaded heterotopic heart transplantation

Two-day-old donor hearts were implanted into the peritoneal cavity of 7-week-old C57BL/6 mice ($n=18$) in an unloaded configuration as previously described [12]. The ascending aorta was anastomosed to the infrarenal aorta, and the pulmonary artery was anastomosed to the infrarenal vena cava (Fig. 2). In rats, this model has previously been shown to receive a preload of approximately 5% of the circulating blood volume to the transplanted heart [11]. Perfusion of the heart occurs via the coronary arteries to the coronary sinus and into the right atrium.

2.4. In vivo imaging of heterotopic heart implants

Two weeks or 3 months postimplantation, mice underwent echocardiographic assessment with pulsed-wave Doppler ultrasound (30 MHz; VisualSonics Inc.). Anesthesia was induced with 1.5% isoflurane (Baxter, Deerfield, IL) vaporized with oxygen at a flow rate of 1 L/min. To eliminate poor visualization due to intestinal gas or rotation of the implanted heart, the midline incision was reopened to eviscerate the intestines and