Available online at www.sciencedirect.com

SciVerse ScienceDirect

Neuromuscular Disorders 22 (2012) 1006–1014

www.elsevier.com/locate/nmd

Cardiomyopathy in the dystrophin/utrophin-deficient mouse model of severe muscular dystrophy is characterized by dysregulation of matrix metalloproteinases

Dawn A. Delfín [a], Kara E. Zang [a], Kevin E. Schill [a], Nikita T. Patel [a], Paul M.L. Janssen [b], Subha V. Raman [c], Jill A. Rafael-Fortney [a,*]

[a] Department of Molecular and Cellular Biochemistry, The Ohio State University, College of Medicine, Columbus, OH 43210, United States
[b] Department of Physiology and Cell Biology, The Ohio State University, College of Medicine, Columbus, OH 43210, United States
[c] Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University, College of Medicine, Columbus, OH 43210, United States

Received 29 September 2011; received in revised form 30 April 2012; accepted 3 May 2012

Abstract

Cardiomyopathy is a significant component in Duchenne muscular dystrophy. Although *mdx* mice are deficient in dystrophin, they only develop mild indicators of cardiomyopathy before 1 year-of-age, making therapeutic investigations using this model lengthy. In contrast, *mdx* mice also lacking utrophin (*utrn*$^{-/-}$*;mdx*) show severely reduced cardiac contractile function and histological indicators of cardiomyopathy by 8–10 weeks-of-age. Here we demonstrate that *utrn*$^{-/-}$*;mdx* mice show a similar pattern of cardiac damage to that in dystrophic patients. Matrix metalloproteinases required for ventricular remodeling during the evolution of heart failure are upregulated in *utrn*$^{-/-}$*;mdx* mice concurrent with the onset of cardiac pathology by 10 weeks-of-age. Matrix metalloproteinase activity is further dysregulated due to reduced levels of endogenous tissue inhibitors and co-localizes with fibroblasts and collagen I-containing scars. *utrn*$^{-/-}$*;mdx* mice are therefore a very useful model for investigating potential cardiac therapies.

Keywords: Cardiomyopathy; Heart failure; Duchenne muscular dystrophy; *mdx*; Utrophin; Matrix metalloproteinases; MMPs; Tissue inhibitors of matrix metalloproteinases; TIMP; Remodeling; MMP-9; TIMP-2

1. Introduction

Ninety-five percent of Duchenne muscular dystrophy (DMD) patients develop cardiomyopathy due to a lack of dystrophin protein in the heart, and greater than 25% die from heart failure [1–3]. As treatments to ameliorate the skeletal muscle pathology in DMD improve, it is expected that heart failure will account for an even higher proportion of DMD deaths [4,5]. Although heart failure is an end-stage disease that can result from a wide variety of primary causes [6], a number of common features usually characterize heart failure progression, including remodeling of heart tissue, ventricular hypertrophy, cardiomegaly, and upregulation of matrix metalloproteinases (MMPs) [7–9]. Although the skeletal muscle pathogenesis associated with DMD has been extensively characterized, the cardiomyopathy and progression to heart failure has not been as well studied. Defining whether the pathogenesis of heart failure associated with muscular dystrophies shares features with heart failure from other causes will have direct implications for current treatment and in the future as new treatment options are developed.

A dystrophin-deficient mouse model of DMD, the *mdx* mouse, shows moderate cardiomyopathy and functional

* Corresponding author. Address: 410 Hamilton Hall, Department of Molecular and Cellular Biochemistry, The Ohio State University College of Medicine, 1645 Neil Ave., Columbus, OH 43210, United States. Tel.: +1 614 292 7043; fax: +1 614 292 4118.
E-mail address: rafael-fortney.1@osu.edu (J.A. Rafael-Fortney).

0960-8966/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.nmd.2012.05.002

cardiac impairment, as measured by contractile response in isolated muscles, by 8–10 weeks of age [10,11]. However, these mice do not demonstrate physiological indicators of heart failure early in life. Utrophin, a homolog of dystrophin, partially compensates for the lack of dystrophin in *mdx* mice and prevents extensive degeneration of cardiac and skeletal muscle. Mice deficient for both dystrophin and utrophin (*utrn*$^{-/-}$;*mdx*, or "dko") have severe cardiomyopathy [11–13] and display the physiological indicators of end-stage heart failure by 8–10 weeks, including a reduction in force development and impairment of relaxation, a negative force-frequency relationship, and a severely blunted β-adrenergic response [10]. A thorough characterization of the cardiomyopathy in these mice will increase the usefulness of this animal model for research into treatments and diagnostics for muscular dystrophy-associated heart failure.

The study of matrix metalloproteinase (MMP) dysregulation in cardiovascular disease is an ever-growing field of research. Since the early 1990s, a large number of studies have shown that one or more members of this enzyme family are dysregulated (usually upregulated) in cardiovascular events leading to heart failure [14–18]. This dysregulation is observed in both patients and animal models. MMPs, especially the gelatinases MMP-2 and MMP-9, proteolyze the extracellular matrix (ECM) in the heart. The ECM-degrading activity of MMPs permits the remodeling process observed in heart failure evolution to occur [7]. Subsequent deposition of connective tissue leads to deleterious fibrosis, resulting in stiffening of the heart and reduced contractile capacity [19]. Studies have shown that MMP-2 or MMP-9 deficiency results in reduced cardiac damage [14,19–22], except in the case of virally-induced cardiomyopathy [23].

MMPs are regulated on multiple levels: changes in gene expression of the proenzyme; activation of the enzyme by cleavage of the inhibitory amino-terminal peptide sequence; and regulation of expression of endogenous tissue inhibitors of MMPs (TIMPs), proteins that strongly inactivate MMPs [22,24]. It is evident that MMP regulation is controlled through multiple mechanisms, which is in accordance with their critical roles in normal physiology and disease-related pathology. Although clinical treatments targeting MMPs have been elusive thus far, research to identify MMP-inhibitory drugs is ongoing, and MMPs may present practical targets in treating heart failure in the future [25].

MMPs have been shown to play an important role in skeletal muscle inflammation and fibrosis present in muscular dystrophy [26–33]. Genetic or pharmacological inhibition of MMP-2 or MMP-9 in *mdx* mice alters skeletal muscle regenerative capacity [27,31]. Serum MMP-9 has also recently been demonstrated to be a biomarker of DMD disease progression in patients [34]. Recently, studies in old *mdx* mice have shown that increased levels of cardiac MMP-2 and MMP-9 correlates with increased cardiac pathology [35,36]. Because of the clear importance of MMPs in general cardiovascular disease, in muscular dystrophy heart and skeletal muscle pathology, and their promise as drug targets for end-stage heart failure, analyzing MMP levels and activities in the more clinically relevant dko mouse model is necessary to better characterize its cardiac pathology, and indicate whether it could be useful for testing therapeutic strategies for DMD cardiac involvement. We hypothesized that increased MMP expression in the hearts of dko mice would correspond to their more severe cardiac pathology compared to *mdx* mice. We therefore analyzed the levels and localization of the gelatinases MMP-2 and MMP-9, and TIMP-1 and TIMP-2 levels in dko hearts compared to *mdx* and normal hearts.

2. Materials and methods

2.1. Animals

The protocol was approved by the Institutional Animal Care and Use Committee at The Ohio State University. *utrn*$^{+/-}$;*mdx* mice were bred to produce *utrn*$^{+/+}$;*mdx* (*mdx*) mice and *utrn*$^{-/-}$;*mdx* (dko) littermates [37]. Wild-type C57BL/10 (C57) mice were maintained as a separate inbred colony. Both sexes of mice were used in equivalent numbers between groups. Breeding cages are maintained on a high-fat breeding diet and mice are fed a standard diet after weaning.

2.2. Tissue preparation

Hearts from male and female mice at 10 weeks were removed and segmented in three pieces from base to apex. The middle segment containing only ventricular tissue was further split into two pieces and each piece was homogenized in a different buffer. For immunoblotting, protein homogenates in Newcastle buffer (4 M urea, 75 mM Tris, pH 6.8, 3.8% SDS) were used. For zymography, homogenates in a non-denaturing buffer with protease inhibitors (100 mM Tris, pH 6.8, 200 mM NaCl, 100 mM $CaCl_2$, 1% Triton X-100, 500 μM PMSF, 500 μM benzamidine, 250 μg/ml leupeptin, 0.1 U/ml aprotinin) were used. Total protein concentrations were quantified using the Dc Protein Assay (Bio-Rad, Hercules, CA).

The remainder of the heart was frozen in blocks in O.C.T. (Tissue-Tek, Torrance, CA) on liquid nitrogen-cooled isopentane. Cryosections (8 μm) were cut serially from blocks for *in situ* zymography, immunofluorescence, and histology.

2.3. Human cardiac magnetic resonance imaging (MRI)

The patient cardiac MRI was obtained with informed consent and approved by the Biomedical Sciences Institutional Review Board of The Ohio State University. *In vivo* human cardiac magnetic resonance imaging (MRI) was performed using the late gadolinium enhancement (LGE) technique [38] in a 15 year-old male with Duchenne muscular