Dystrophin-deficient myocardium is vulnerable to pressure overload in vivo

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Received 6 July 2000; accepted 2 January 2001

Abstract

Objective: Dystrophin provides mechanical reinforcement to the membranes of myocytes. Dystrophin abnormalities are known to cause cardiomyopathy and skeletal muscle disorders; however, the pathogenesis of these abnormalities remains unclear. Dystrophin-deficient skeletal muscle is vulnerable to stresses such as stretch and hypo-osmotic shock. We investigated whether the myocardium of dystrophin-deficient (mdx) mice shows increased vulnerability to acute pressure overload in vivo.

Methods and results: Abdominal aortic banding was performed in 12-week-old mdx and control mice. The aortic pressure was measured by cannulation of the right carotid artery at the time of sacrifice. Systolic pressures in mdx mice at 0, 1, 2, 7 and 14 days after aortic banding were 100±11, 119±7, 123±4, 134±11 and 130±10 mmHg, respectively. Microscopic analysis revealed focal lesions in the left ventricular wall in banded mdx mice. These lesions consisted of damaged myocytes and inflammatory cells, and also of fibrosis at a late stage. Similar lesions were not observed in non-banded or banded control mice. The proportion of areas of lesions to total left ventricular area increased over time: 1.0±0.6% in mdx mice without aortic banding (sham, n=6), and 1.7±1.4% 1 day (n=6, vs. sham, NS), 2.6±1.9% 2 days (n=7, vs. sham, P<0.05), 6.3±6.5% 7 days (n=13, vs. sham, P<0.05) and 9.9±8.3% 14 days after aortic banding (n=15, vs. sham, P<0.01). Furthermore, linear regression analysis revealed a significant correlation between percentage of lesion area and systolic pressure in mdx mice (P<0.05).

Conclusion: Dystrophin-deficient myocardium is more vulnerable than normal myocardium to pressure overload in vivo. This result has two clinical implications: (1) the patients with dystrophynopathy, such as the Duchenne and the Becker types of muscular dystrophy and X-linked type of dilated cardiomyopathy, who develop arterial hypertension should be treated aggressively, and (2) they should avoid stresses that elevate blood pressure. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Blood pressure; Cardiomyopathy; Histo(patho)logy; Hypertension; Myocytes

1. Introduction

Dystrophin is an important cytoskeletal component of striated muscle cells, in which it provides structural support to the plasma membranes [1–4]. Although the functional role of dystrophin is not completely understood, immunohistochemical analysis has demonstrated that this protein is localized in the sarcolemma [5]. Genetically transmitted abnormalities in the expression of this protein are complex and result in three clinically distinct disorders: the Duchenne (DMD) and the Becker (BMD) types of muscular dystrophy [6,7] and X-linked type of dilated cardiomyopathy [8].

The main role of the cytoskeleton is to provide the major structural framework to maintain cellular integrity. Dystrophin is thought to link between the subsarcolemmal cytoskeleton and the extracellular matrix. The N-terminal domain of dystrophin binds to F-actin in the cytoskeleton [9], whereas the C-terminal region interacts with a set of transmembrane- and membrane-associated proteins, including α, β-dystroglycans, α, β, γ, δ-sarcoglycans, syntrophins, sarcospan and dystrobrevin [10–19]. α-Dystroglycan binds to laminin-2 (merosin) in the overlying basal lamina [11,15]. The findings that abnormalities in dystrophin [1,2,6–8], actin [20], merosin [21], and some of
the dystrophin–glycoprotein complex [22–25] are linked to skeletal and/or cardiac muscle dystrophy in humans and in animal models, points to the importance of the appropriate transfer of extracellular stimuli to the interior of the cell to maintain sarcolemmal membrane stability. This concept is in accord with the observation that the lack of integrin α7, which also transduces the extracellular signal to the interior of the cells via focal adhesion kinase, causes muscular dystrophy in mice [26].

Damage to both cardiac and skeletal muscle occurs in the majority of patients with DMD and BMD [25]. Furthermore, several studies have demonstrated that dystrophin-defected skeletal muscle cells are abnormally vulnerable to stretch [27,28] and hypoosmolarity [29,30]. However, no information is available on the susceptibility of dystrophin-deficient cardiac muscle to the damage induced by mechanical forces related to pressure overload. Accordingly, the present study was designed to compare the extent of cardiac damage following aortic banding in dystrophin-deficient and control mice.

2. Methods

2.1. Animals and surgical procedure

Twelve-week-old male dystrophin-deficient (mdx) mice and control (C57BL/10ScSn) mice were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and diethylether. The aorta was exposed via a midline abdominal incision under a dissecting microscope. The abdominal aorta was banded with 6-0 silk thread between the right and left renal arteries using a 26-gauge needle (outside diameter, 0.45 mm) to standardize the diameter of the ligation [31,32]. Sham operation was performed in controls using an identical procedure, except for the ligation. This study was carried out in accordance with the Guide for Animal Experimentation, Faculty of Medicine, Kagoshima University and conforms with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Measurement of aortic pressure

At 1, 2, 7, and 14 days after aortic banding, the right carotid artery was cannulated with a flame-stretched polyethylene tube (SP45; Natume, Tokyo, Japan) and connected to a polygraph system (Nihon Kohden, Tokyo, Japan) for measurement of the aortic pressure.

2.3. Microscopic analysis

After measurement of the aortic pressure, each mouse was sacrificed, the heart was excised and the atria removed. The ventricles were divided into the left ventricle, including the intraventricular septum, and right ventricular free wall. They were rinsed with HEPES buffer, weighed, fixed in neutral-buffered 10% formalin and embedded in paraffin. The tissue sections were stained with hematoxylin–eosin and Azan. Left ventricular hypertrophy was assessed by measurements of: (1) the ratio of left ventricular wet weight to body weight, and (2) the diameters of the myocytes (n=1000) in the area of the nucleus. To determine the degree of myocardial damage, three cross-sectional cuts of the left ventricle (apex, mid and basal portion) were selected and stained with Azan, and the percentage of the sum of the areas of lesion to the total area of the tissue was calculated using a computerized digital image analysis system (NIH image, NIH Research Serviced Branch) by two blinded observers. Three measurements were averaged.

2.4. Statistical analysis

All values are expressed as mean±S.D. Statistical comparison of aortic banded mice with sham-operated mice was performed with the Student’s t-test for unpaired values. Linear regression analysis was performed to assess a correlation between aortic pressure and percentage of lesion area. P values <0.05 were considered statistically significant.

3. Results

3.1. Mortality

The acute operative mortality was <20%, with the mortality for the mice that recovered from anesthesia being <10%. No significant difference in mortality existed between control and mdx mice.

3.2. Aortic pressure and left ventricular hypertrophy

Aortic pressure increased significantly 24 h after abdominal aortic banding in both control and mdx mice, but was not significantly different between the two groups (Fig. 1). Left ventricular hypertrophy was observed in the both groups at 14 days after aortic banding. The left ventricular weight to body weight ratio and the mean myocyte diameter in the area of the nucleus were significantly increased. There was no significant difference in the degree of the ventricular hypertrophy between in mdx and in control (Table 1).

3.3. Histopathology

Microscopic observation revealed no myocyte damage, inflammatory cell infiltration or fibrosis in control mice with or without aortic banding. Lesions were scarcely found in sham-operated mdx mice (Fig. 2A–C). In mdx
of left ventricle was observed (Fig. 2G). Mild interstitial fibrosis was observed at a late stage in control mice [36], and triiodothyronine (T3) treatment induced more damage in dystrophin-deficient muscles from both mdx mice and DMD patients in vitro. Petrof et al. [27] and Moens et al. [28] have reported that skeletal muscle from mdx mice is damaged by contractions with stretch in vitro to a greater extent than is muscle from control mice. Carter et al. [33] and Vilquin et al. [34] have demonstrated that excessive running exercise leads to muscle damage in mdx mice. Furthermore, myotubes cultured from mdx mice show an increased susceptibility to oxidative stress induced by reactive oxygen species [35]. Injection of purified mast cell granules into the gastrocnemius muscle induced widespread myofiber necrosis in mdx mice [36], and triiodothyronine (T3) treatment induced more damage in both cardiac and skeletal muscles in mdx mice than in control mice [37]. These data and the observations in the present study suggest that dystrophin protects the muscle cells against the membrane damage induced by several stresses, especially by mechanical stress.

The present study is the first to describe the increased vulnerability of dystrophin-deficient cardiac myocytes in vivo to increased aortic pressure. We were unable to clarify the mechanism which renders dystrophin-deficient myocardium vulnerable to pressure overload in vivo. However, plausible mechanisms have been proposed. Intracellular Ca$^{2+}$ has been reported to be elevated in skeletal muscle from mdx mice, and this change is associated with a high susceptibility to damage by several stresses.

4. Discussion

The present study demonstrates that the dystrophin-deficient myocardium of mdx mice is more vulnerable than normal myocardium to pressure overload in vivo and that the degree of injury correlates with the level of systolic pressure. These findings are in accord with the concept that dystrophin provides mechanical reinforcement to the sarcolemmal membrane of cardiac myocytes.

Several studies have demonstrated the weakness of dystrophin-deficient muscle. Menke and Jockusch [29,30] have noted decreased osmotic stability of dystrophin-deficient muscles from both mdx mice and DMD patients in vitro. Petrof et al. [27] and Moens et al. [28] have reported that skeletal muscle from mdx mice is damaged by contractions with stretch in vitro to a greater extent than is muscle from control mice. Carter et al. [33] and Vilquin et al. [34] have demonstrated that excessive running exercise leads to muscle damage in mdx mice. Furthermore, myotubes cultured from mdx mice show an increased susceptibility to oxidative stress induced by reactive oxygen species [35]. Injection of purified mast cell granules into the gastrocnemius muscle induced widespread myofiber necrosis in mdx mice [36], and triiodothyronine (T3) treatment induced more damage in both cardiac and skeletal muscles in mdx mice than in control mice [37]. These data and the observations in the present study suggest that dystrophin protects the muscle cells against the membrane damage induced by several stresses, especially by mechanical stress.

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Table 1

<table>
<thead>
<tr>
<th>Hemodynamic and hypertrophic parameters in control and mdx mice*</th>
<th>Control mice</th>
<th>mdx mice</th>
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<tr>
<td></td>
<td>Sham (n=9)</td>
<td>14 days after aortic banding (n=13)</td>
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<tr>
<td>Systolic pressure (mmHg)</td>
<td>108±9</td>
<td>137±13***</td>
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<tr>
<td>Left ventricular weight (mg)</td>
<td>3.2±0.1</td>
<td>3.5±0.2**</td>
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<tr>
<td>Body weight (g)</td>
<td>11.5±0.4</td>
<td>12.8±0.4**</td>
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<td>Cell diameter (μm)</td>
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* Data are presented as mean±S.D. Cell diameter: mean cell diameter in the area of the nucleus (n=1000). * P<0.05, ** P<0.005, *** P<0.0001 vs. sham-operated mice.
rate of protein degradation [38]. In addition, electrophysiologic studies have revealed that the open probability of Ca$^{2+}$ leak channels is markedly increased in dystrophic myotubes [39], and that myotubes lacking dystrophin possess a novel type of mechano-transducing channel [40]. These may contribute to the elevation of the intracellular levels of Ca$^{2+}$ in muscle of mdx mice and patients with DMD. Another possibility is induction of apoptosis in cardiomyocytes. Gottlieb et al. [41] first reported that cardiomyocytes underwent apoptosis after reperfusion injury. Then, apoptotic cardiomyocytes were found in various cardiac diseases, including ischemic and non-ischemic heart failure, myocardial infarction, myocarditis, and arrhythmias [42]. In addition, it is reported that...
dystrophies patients compared with age-matched controls. Since the localization and extent of lesions in delta-sarcoglycan knockout mice [46] is similar to those in our study, it is possible that pressure overload induced cardiac lesions may be due to coronary vascular dysfunction. Further examination will be needed.

In the present study we have utilized dystrophin-deficient (mdx) mice, which have genotypic abnormalities identical to those in patients with DMD. These abnormalities involve a nonsense mutation in the dystrophin gene [48] and result in the absence of the dystrophin polypeptide. mdx mice show histologic signs of muscular dystrophy during the first 6 weeks of life [49]; however, their subsequent clinical course differs markedly from that of patients with DMD. The mdx mice show little weakness in skeletal or cardiac muscle, and have an almost normal life span. On histologic examination, cardiac muscle from mdx mice reveals few lesions at 8 weeks of age. Nevertheless, some small lesions are present after 9 weeks (unpublished data). One possible explanation for the clinical differences between mdx mice and DMD patients is that structurally related proteins compensate for the lack of dystrophin more effectively in mdx mice than in DMD patients. Utrophin, a dystrophin-related protein, is upregulated in mdx mice [50], and expression of this protein has been shown to attenuate the dystrophic changes in these animals. Mice lacking both dystrophin and utrophin develop skeletal and cardiac myopathies [51]. Even mdx mice developed a cardiomyopathy by pressure overload in the present study.

In conclusion, dystrophin-deficient myocardium is vulnerable to pressure overload in vivo. This result has two clinical implications: (1) the patients with dystrophopathy, such as DMD, BMD and X-linked type of dilated cardiomyopathy, who develop arterial hypertension should be treated aggressively, and (2) they should avoid stresses that elevate blood pressure.

Acknowledgements

This study was presented in part at the 70th Scientific Sessions of the American Heart Association, Orlando, FL, 1997 and supported in part by the Scientific Research Grant from the Ministry of Education, Science and Culture of Japan.

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