Early Treatment With Lisinopril and Spironolactone Preserves Cardiac and Skeletal Muscle in Duchenne Muscular Dystrophy Mice

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Background—Nearly universal cardiomyopathy in Duchenne muscular dystrophy (DMD) contributes to heart failure and death. Because DMD patients show myocardial fibrosis well before functional impairment, we postulated that earlier treatment using drugs with antifibrotic effect may be beneficial.

Methods and Results—Three groups of 10 utrn+/−;mdx, or “het” mice, deficient for dystrophin and haploinsufficient for utrophin with skeletal myopathy and cardiomyopathy that closely mimics clinical DMD were studied. One het group received spironolactone and lisinopril starting at 8 weeks of life (het-treated-8); a second received the same starting at 4 weeks of life (het-treated-4), and the third het group was untreated. At 20 weeks, all mice had normal ejection fractions though circumferential strain rate was abnormal (−0.21±0.08) in untreated hets. This improved to −0.40±0.07 in het-treated-8 mice (P=0.003) and further improved to −0.56±0.10 in het-treated-4 mice (P=0.014 for het-treated-4 versus het-treated-8). Treated mice showed less cardiomyocyte damage, with a 44% reduction in intracardiomyocyte serum immunoglobulin G localization in het-treated-8 mice (P<0.0001) and a further 53% reduction in het-treated-4 mice (P=0.0003 versus het-treated-8); matrix metalloproteinases were similarly reduced. Cardiac, limb, and diaphragm function by ex vivo muscle testing remained at 80% of normal with early treatment compared to a decline to 40% of normal skeletal muscle function without treatment.

Conclusions—These findings offer clinically available medications with proven antifibrotic effect as a new therapeutic strategy in DMD. Early initiation greatly attenuated myocardial disease and, for the first time with these drugs, improved skeletal myopathy. Thus, early initiation of such agents warrants further clinical evaluation to maintain ambulatory, respiratory, and cardiac function for patients with DMD and related myopathies. (Circulation. 2011;124:582-588.)

Key Words: cardiomyopathy ■ muscles ■ aldosterone antagonists

Inherited myopathies produce progressive immobility due to limb muscle degeneration, respiratory failure due to diaphragm involvement, and cardiomyopathy due to myocardial disease.1 The most common form is Duchenne muscular dystrophy (DMD), an X-linked disorder that leads to absence of the sarcolemmal protein dystrophin and impairs ambulation beginning in children age 3 to 7 years. Duchenne muscular dystrophy patients universally develop cardiomyopathy by the third decade of life, and present guidelines advocate periodic screening with echocardiography to detect LV systolic dysfunction, particularly reduced LV ejection fraction (LVEF).2 Use of drugs like aldosterone antagonists that have antifibrotic effects are only advocated for treatment of patients with advanced heart failure.4 Recent evidence supports initiation earlier in the progression of cardiac disease in symptomatic patients with evident LV dysfunction.5

Clinical Perspective on p 588

Our present study was motivated by increasing evidence suggesting that myocardial disease is developing in DMD patients well before LVEF becomes abnormal.6 Observing that the early histopathological changes that occur in both skeletal and cardiac muscle invariably leads to fibrosis, we...
postulated that drugs with an antifibrotic effect may be most beneficial if started earlier in the disease course. Therefore, we implemented a prospective blinded study to test the hypothesis that early versus late treatment with clinically available ACE inhibitors and aldosterone antagonists provides significantly greater myocardial protection in a mouse model of DMD. In addition, because of (1) the reported antifibrotic properties of spironolactone and lisinopril, (2) the absence of any published investigations of these drugs on skeletal muscles in any muscular dystrophy model, and (3) the presence of large amounts of fibrosis in het mice, we decided to include analysis of skeletal muscle effects in addition to cardiac effects of the drug treatment in our studies.

Methods

All protocols were approved by the institutional laboratory animal care and use committee. For this study we used a mouse model deficient for dystrophin that is also haploinsufficient for its partially deficient for dystrophin that is also haploinsufficient for its partially compensating homolog utrophin, utrn ("het") mice, because we have previously shown that their skeletal muscle fibrosis is more severe than that of mdx mice, making it a more accurate phenotypic model of DMD. Het mice were bred and genotyped as previously described.10 Groups of 10 of these mice, housed 2 to 3 to a cage, were given water bottles containing 66 mg/L lisinopril (Sigma L-8494) in reverse-osmosis water, starting at 4 or 8 weeks of age, or were given water bottles containing 6292) ethanol) in reverse-osmosis water, starting at 4 or 8 weeks of age, or were given water bottles containing 6292) ethanol) in reverse-osmosis water, starting at 4 or 8 weeks of age, or

One day after in vivo CMR examination, ECGs were recorded and the mice were then euthanized. In vitro cardiac muscle function was assessed as previously described in detail for a related mouse model.12 Briefly, small, linear, multicellular preparations were dissected and electrocardiographically recorded and analyzed to determine mechanical responses under baseline and calcium conditions. In addition to baseline function, the main 3 mechanisms (length-dependent activation, frequency-dependent activation, and β-adrenergic stimulation) that regulate in vivo myocardial force development were assessed. Isolated strips of diaphragm muscle were assessed on their force-generating ability whereas maximal tetanic strength of the extensor digitorum longus muscle was assessed as well as its susceptibility to repetitive eccentric stress were assessed as previously described.13,14

The remainder of the heart tissue and skeletal muscles including diaphragm and quadriceps were embedded in optimal-cutting-temperature medium and frozen on liquid-nitrogen cooled isopentane for subsequent histological analyses. Eight μm cryosections were stained by hematoxylin and eosin by standard methods or stained for intracellular immunoglobulin G (IgG). IgG immunostaining was performed using a CY3-conjugated antimouse IgG antibody (1:100) (Jackson ImmunoResearch Laboratories 115-165-146) as previously described with or without containing with anti-Collagen I or anti-ERTR7 antibodies (Abcam ab7778 and ab51824, respectively). The percentage of IgG-stained pixels in composites of photomicrographs that represented the majority of heart or quadriceps sections from each mouse were quantified using Image J. For in situ zymography, heart cryosections were fixed and incubated for 9 hours at 37°C with a solution of gelatin conjugated to Oregon Green 488 (Invitrogen Molecular Probes) then washed with 10 mmol/L EDTA to stop activity. Summary values are presented as mean±SE.

One-way ANOVA followed by posthoc test using Bonferroni adjustment, where applicable, was used to determine statistically significant differences.

Results

Cardiovascular magnetic resonance showed normal LVEF in all 3 groups. Strain analysis, however, showed measurable differences in both systolic and diastolic function (Figure 1);
these were more significant in basal LV segments compared to the apex, similar to DMD cardiomyopathy in humans that initially demonstrates abnormalities in the base versus the apex of the LV.16 Mean systolic circumferential strain rate measured in a short-axis cine view at the base of the heart was \(0.21 \pm 0.08\) in untreated het mice, improved to \(-0.40 \pm 0.07\) in het mice whose treatment was initiated at 8 weeks of life, and improved even more to \(-0.56 \pm 0.10\) in het mice whose treatment was initiated at 4 weeks of life \(P=0.003\) for het-untreated versus het-treated-8; \(P<0.0001\) for het-untreated versus het-treated-4, and \(P=0.014\) for het-treated-4 versus het-treated-8). Diastolic strain measurement showed similar improvements: \(0.24 \pm 0.09\) in untreated hets, \(0.42 \pm 0.10\) in hets whose treatment began at 8 weeks, and \(0.59 \pm 0.08\) in hets whose treatment began at 4 weeks \(P=0.007\) for het-untreated versus het-treated-8, \(P<0.0001\) for het-untreated versus het-treated-4, and \(P=0.012\) for het-treated-4 versus het-treated-8).
Electrocardiographic recordings obtained in conscious unrestrained mice showed no significant difference in heart rate among C57BL/10 controls, het-untreated, het-treated-8 and het-treated-4 mice ($P > 0.19$), nor were there significant differences across groups in QT interval ($P > 0.31$).

In vitro cardiac muscle force and response to isoproterenol also showed a clear trend toward improvement in the het-treated-4 group (Figure 2A through F) at 20 weeks of age compared to untreated het mice. The profound cardiac damage present in untreated het mice was almost completely prevented by the drug treatment in both groups of treated mice (Figure 3A). Degenerating cardiomyocytes, as detected by intracellular serum IgG localization were prevalent throughout the left ventricle of untreated het mice (Figure 3A). These were reduced 44% in het-treated-8 mice ($P < 0.0001$ versus het-untreated mice) and showed a further 53% reduction from these levels in the het-treated-4 group ($P < 0.0001$ versus het-untreated mice; $P = 0.0003$ versus het-

**Figure 3.** Drug treatment improves histological parameters of heart and skeletal muscles. A, Hematoxylin and eosin (H&E)-stained LV sections show the cardiac damage prevalent throughout het-untreated hearts that is almost completely prevented in both treatment groups. Intracellular localization of mouse IgG (green) indicates damaged myocardium that is significantly attenuated in het-treated-8 and even further improved in het-treated-4 hearts. Gelatinase in situ zymography (ISZ) shows the combined activity of matrix metalloproteinases 2 and 9 (bright green), indicative of ventricular remodeling, that is also attenuated in the het-treated-8 hearts and almost entirely prevented in the het-treated-4 hearts. B, Immunoglobin G localization (green) in quadriceps skeletal muscle sections indicates a profound and significant reduction of ongoing myofiber damage in the het-treated-4 group, with intermediate effects in the het-treated-8 group compared to untreated hets. Localization of collagen I (red) in the matrix surrounding individual muscle fibers is shown to demonstrate the intracellular localization of the IgG staining. Bar=50 μm. Het-untreated indicates $utm^{+/-};mdx$ mice untreated with spironolactone and lisinopril; Het-treated (8), $utm^{+/-};mdx$ mice treated with spironolactone and lisinopril at 8 weeks; and Het-treated (4), $utm^{+/-};mdx$ mice treated with spironolactone and lisinopril at 4 weeks; H&E, hematoxylin and eosin; IgG, immunoglobulin G; and ISZ, in situ zymography.

**Figure 4.** A, The average percentage (±SE) of section area stained for mouse IgG for heart (left) and quadriceps skeletal muscles from het-untreated and treated groups shows significant reductions in ongoing muscle damage. B, Blinded visual scoring of gelatinase activity from in situ zymography supports reductions of matrix metalloproteinase remodeling in treated groups. *$P < 0.03$ versus untreated, **$P < 0.05$ versus 8-week. Panel A, $n = 7$ to 10 per group. Panel B, $n = 5$ per group. IgG indicates immunoglobulin G; Het-untreated, $utm^{+/-};mdx$ mice untreated with spironolactone and lisinopril; Het-treated (8), $utm^{+/-};mdx$ mice treated with spironolactone and lisinopril at 8 weeks; and Het-treated (4), $utm^{+/-};mdx$ mice treated with spironolactone and lisinopril at 8 weeks; and ISZ, in situ zymography.
treated-8) (Figure 4). Drug treatment also prevented matrix metalloproteinase gelatinase activity, a key indicator of ventricular remodeling (Figure 3) and the infiltration of activated fibroblasts that secrete both matrix metalloproteinases and collagen (Figure 5).

Surprisingly, het mice started on drug treatment at 4 weeks of age showed a dramatic improvement in both diaphragm and extensor digitorum longus muscle function compared to isogenic normal (C57BL/10) control muscle force compared to 40% of normal force in untreated het mice, (Figure 2G, extensor digitorum longus: P=0.0006 C57BL/10 versus het-untreated mice; P=0.0067 C57BL/10 versus het-treated-8; P=0.013 het-untreated versus het-treated-4; Figure 2I, diaphragm: P=0.0017 C57BL/10 versus het-untreated; P=0.0086 C57BL/10 versus het-treated-8; P=0.044 het-untreated versus het-treated-4).

Limb muscles demonstrated a similar reduction of ongoing muscle degeneration as shown in the heart (Figure 3B), with a 2-fold reduction in the het-treated-4 group (P=0.025 het-untreated versus het-treated-4) (Figure 4). However, gross limb muscle histopathology was similar in all groups. Despite the profound functional improvement in diaphragm conferred by early drug treatment initiation in het-treated-4 muscles, the diaphragm showed no obvious improvement in any histopathological parameter assessed (not shown).

**Discussion**

We show for the first time in a mouse model of DMD a remarkable protective effect on both cardiac and skeletal muscle of very early initiation of treatment with an aldosterone antagonist plus an ACE inhibitor. Treatment was initiated well before evident cardiac dysfunction, and even untreated animals at 20 weeks of life still showed preserved EF despite extensive myocardial fibrosis and injury. Despite genetics that would otherwise dictate inevitable cardiomyopathy and skeletal muscle disease, early treatment with spironolactone in combination with lisinopril provided near normalization of muscle function and considerable prevention of tissue changes.

Gross histopathology of limb muscle was similar in all groups despite less muscle degeneration by IgG staining and matrix metalloproteinase activity; this may be explained by ongoing damage in dystrophic skeletal muscle at 4 weeks of age when such damage is not yet evident in cardiac muscle. Also, despite dramatic improvement in diaphragm function, there was no appreciable histopathological improvement in diaphragm. This result is likely due to the well documented crisis period of damage that occurs in dystrophin-deficient mouse diaphragm between 3.5 and 4 weeks of age, before the initiation of treatment in either group, and supports that treatment preceding damage likely results in the most improvement in all parameters.

Numerous pharmacological treatment approaches have been investigated to attenuate the devastating sequelae of cardiomyopathy and skeletal myopathy in DMD, though none before this work have shown such remarkable efficacy for both myocardial and skeletal muscle disease. A recent critical review of the literature listed therapeutics with sufficient evidence for clinical use, and endorsed the use of glucocorticoids that limit decline in muscle strength and function. However, long-term steroid therapy brings a wide range of attendant complications. Further, glucocorticoids have shown inconsistent effects on myocardial disease, ranging from modest protection and reduced fibrosis to accelerated progression and even increased fibrosis. Standard heart failure medications such as ACE inhibitors and β-blockers are advocated once LVEF is abnormal by echocardiography, though evidence suggests that changes in myocardial structure and function are well underway even with normal LVEF using more sensitive CMR-based strain and fibrosis imaging techniques.

Mechanistic insights from this work include evidence supporting that the drugs’ benefit is likely not mediated via inhibition of fibroblast proliferation and migration. As precise molecular pathways are better defined through ongoing preclinical studies, these results behoove initiation of clinical trials to evaluate the potentially significant therapeutic benefits across a broad range of genetic and acquired cardiomyopathies. Any myocardial disease marked by fibrosis, from hypertensive heart disease to diabetic cardiomyopathy, warrants evaluation of earlier use of aldosterone antagonism than what is presently advocated. Similarly, our findings of these drugs’ effect on attenuating damage and maintaining strength of skeletal muscle suggest potential benefit for conditions such as sarcopenia and cachexia where therapeutic options are presently limited.

The threshold doses for lisinopril and spironolactone were not established in this study but were based on therapeutic doses commonly used in other mouse studies and relate to the allometric scaling needed to account for different pharmacoki-
netics between rodents and humans. Clinical trials testing the
efficacy of these drugs in DMD patients will likely begin at
the comparable per-kilogram body weight doses typically used
clinically. Because both lisinopril and spironolactone were
administered, we cannot determine from these data if the
benefits demonstrated were due to the combination of drugs or
spironolactone alone. Ongoing studies with administration of
each drug individually should address this question.

In conclusion, combining aldosterone antagonism with
ACE inhibition at an extremely early stage of genetically
determined myocardial and skeletal muscle disease poten-
tially offers maximal inhibition of tissue injury and fibrosis
with superior outcomes. Our work indicates that such a
combined approach yields (1) improvements in both skeletal
and cardiac muscle histology, unlike prior investigations that
showed improvements in one tissue but not in another, and
(2) preservation of function to an extent that far exceeds that
of any prior pharmacological therapy regimen studied in
mouse models of DMD.24–26

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Disclosures

None.

References

1. Verhaert D, Richards K, Rafael-Fortney JA, Raman SV. Cardiac
involvement in patients with muscular dystrophies: magnetic resonance
imaging phenotype and genotypic considerations. Circ Cardiovasc

2. English KM, Gibbs JL. Cardiac monitoring and treatment for children
and adolescents with neuromuscular disorders. Dev Med Child Neurol.

3. Bushby K, Muntoni F, Bourke JP. 107th ENMC international workshop:
the management of cardiac involvement in muscular dystrophy and
myotonic dystrophy. June 7–9, 2002, Naarden, the Netherlands.

PS, Silver MA, Stevenson LW, Yancy CW, Antman EM, Smith SC, Jr,
Adams CD, Anderson JL, Faxon DP, Fuster V, Halperin JL, Hiratzka LF,
Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA 2005
guideline update for the diagnosis and management of chronic heart
failure in the adult: a report of the American College of Cardiology/Am-
ERICAN Heart Association Task Force on Practice Guidelines (Writing
Committee to Update the 2001 Guidelines for the Evaluation and Man-
agement of Heart Failure): developed in collaboration with the American
College of Chest Physicians and the International Society for Heart and
Lung Transplantation: Endorsed by the Heart Rhythm Society.
Circulation. 2005;112:e154–e235.

5. Zannad F, McMurray JJ, Krum H, van Veldhuisen DJ, Swedberg K,
Bradshaw C, McMurray JJ, Krum H, van Veldhuisen DJ, Swedberg K,
Shi L, Rahuel M, Fenoglio-Preiser CL, Reuter U, Bueno H, Afroz S,
Zreiqat H, Mohri M, Agler M, Bristow MR, Inzitari D, Lohse CM, Redfield
2010;364:11–21.

6. Hor KN, Wansapura J, Markham LW, Mazar W, Cripe LH, Fleck R,
Benson DW, Gottliebson WM. Circumferential strain analysis identifies
strata of cardiomyopathy in Duchenne muscular dystrophy: a cardiac
magnetic resonance tagging study. J Am Coll Cardiol. 2009;53:
1204–1210.

7. Brilla CG, Funck RC, Rupp H. Lisinopril-mediated regression of myo-

8. Sanderson JE. New treatments for myocardial fibrosis. Cardiovasc

Zhang Y, Yeung L, Wu EB, Chan WW, Wong JT, So N, Yu CM. Aldosterone
receptor antagonist induces reverse remodeling when added to angiotensin
66.

Kaminiski HJ, Liu L, Ransohoff RM. Haplinsufficiency of utrophin gene
worsens skeletal muscle inflammation and fibrosis in mdx mice. J Neurol

11. Hainsey TA, Senapati S, Kuhn DE, Rafael JA. Cardiomyopathic
features associated with muscular dystrophy are independent of dys-
trophy absence in cardiovascular. Neuromuscular Disord. 2003;13:
294–302.

12. Janssen PML, Hiranandani N, Mays TA, Rafael-Fortney JA. Urophin
deficiency worsens cardiac contractile dysfunction present in dystrophin-
deficient mdx mice. Am J Physiol Heart Circ Physiol. 2005;289:
H2373–H2378.

13. Hor KN, Gottliebson WM, Carson C, Wash E, Cnota J, Fleck R,
Wansapura J, Klimczek P, Al-Khalidi HR, Chung ES, Benson DW,
Mazar W. Comparison of magnetic resonance feature tracking for strain
calculation with harmonic phase imaging analysis. J Am Coll Cardiol

14. Peterson JM, Kline W, Canan BD, Ricca DJ, Kaspar BK, Delfin DA,
DiRienzo K, Clemens PR, Robbins PD, Baldwin AS, Flood P, Kaumaya
P, Frietas M, Kornegay JN, Mendell JR, Rafael-Fortney JA, Guttridge
DC, Janssen PML. Peptide-based inhibition of NF-κB rescues diabetes
and myocardial contractile dysfunction in a murine model of Duchenne

15. Rodino-Klapac LR, Janssen PM, Montgomery CL, Coley BD, Chiocone
LG, Clark KR, Mendell JR. A translational approach for limb vascular
delivery of the micro-dystrophin gene without high volume or high
2007;5:45.

16. Puchalski MD, Williams RV, Askovich B, Sower CT, Hor KH, Su JT,
Pack N, Libella E, Gottliebson WM. Late gadolinium enhancement:
precursor to cardiomyopathy in Duchenne muscular dystrophy? Int J Car-

17. Stedman HE, Sweeney HL, Shragg JR, Maguire HC, Panettieri RA,
Petrol B, Narusawa M, Leferovich JM, Sladky JT, Kelly AM. The mdx
mouse diaphragm reproduces the degenerative changes of Duchenne

18. Bushby K, Nunn AL, Binkraft DJ, Case LE, Clemens PR, Cripe LH,
Kaul PA, Frietas M, Kornegay JN, Mendell JR, Rafael-Fortney JA, Guttridge
DC, Janssen PML. Peptide-based inhibition of NF-κB rescues diabetes
and myocardial contractile dysfunction in a murine model of Duchenne

19. Rodino-Klapac LR, Janssen PM, Montgomery CL, Coley BD, Chiocone
LG, Clark KR, Mendell JR. A translational approach for limb vascular
delivery of the micro-dystrophin gene without high volume or high
2007;5:45.

20. Markham LW, Kinnett K, McDonald DA, Cripe LH. Comparison of
magnetic resonance feature tracking for strain calculation with harmonic
phase imaging analysis. J Am Coll Cardiol Cardiovascular Imaging.

Corticosteroid treatment retards development of ventricular dysfunction in

22. Bauer R, Straub V, Blain A, Kinnett K, McDonald D, Pandya S, Poyosky J,
Shapiro I, Tomeszko J, Constantin C, Diagnosis and management of Duchenne
muscular dystrophy: part 1: diagnosis, and pharmacological and psychosocial

23. Manzur AY, Kuntzter T, Pike M, Swan A, Glucocorticoid corticosteroids
for Duchenne muscular dystrophy. Cochrane Database Syst Rev. 2008;
CD003725.

24. Guerra AD, Rawat R, Sali A, Spurgeon CF, Pirzilli E, Cha HJ, Pandey
GS, Gurnadi P, Fanizza DF, Farajian V, Escolar DM, Bossi L, Becker M,
Zer P, de la Porte S, Gordish-Dressman H, Partridge T, Hoffman EP,
Nagaraju K. Functional and molecular effects of arginine butyrate and
prednisone on TGF-beta1 and collagen in diaphragm muscle from mdx

RM. Haplinsufficiency of utrophin gene worsens skeletal muscle inflamma-

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CLINICAL PERSPECTIVE

Duchenne muscular dystrophy (DMD) is a universally fatal disorder, with cardiomyopathy and skeletal myopathy leading to progressive immobility, respiratory impairment, heart failure, and death. Current treatment options are limited to glucocorticoids that produce inconsistent effects on myocardial disease. Guidelines for management of cardiomyopathy advocate medications such as angiotensin-converting enzyme inhibitors and β-blockers only when left ventricular ejection fraction drops below normal. We tested the hypothesis that early treatment using the angiotensin-converting enzyme inhibitor lisinopril in combination with the aldosterone antagonist spironolactone with proven antifibrotic effect might prevent muscle damage and preserve muscle function. We found that these drugs dramatically improved function of cardiac muscle, leg muscle, and diaphragm in utrn+/−:mdx or “het” mice whose skeletal myopathy and cardiomyopathy closely mimics clinical DMD. Furthermore, histology demonstrated striking reduction in cardiomyocyte damage. These results offer clinically available medications as a new therapeutic strategy in DMD. For the first time, these historically cardioprotective drugs were shown to protect skeletal muscle as well. These preclinical results suggest that angiotensin-converting enzyme inhibition plus aldosterone antagonism may deliver improved ambulation, respiratory function, and cardiac status for patients with DMD. Further investigations are also warranted to tests these drugs’ efficacy in attenuating the skeletal myopathy that accompanies a variety of myocardial disorders.
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