Skeletal muscle and cardiac function in B10-mdx mice

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Introduction

Duchenne muscular dystrophy (DMD) is a disease characterized by progressive muscle weakness, eventually leading to heart failure. B10-mdx mice, which lack dystrophin, are widely used as a model of DMD[1]. However, the decline in muscle and cardiac functions is known to be mild[2], and it is thought that the compensatory upregulation of utrophin is one of the contributing factors[3].In this study, the pathological progression by aging of the muscle and cardiac functions was investigated in B10-mdx mice over time. Furthermore, we investigated whether the reduction of utrophin, in addition to dystrophin deficient, exacerbates the pathology.

Highlight of this study

- ✓ The decline in muscle function was detected by the grip test and treadmill test, but not by the rotarod test.
- ✓ Regarding cardiac function, diastolic function declined first, followed by a mild decline in systolic function.
- ✓ *Dmd* ex52 hemi knockout/*Utrn* hetero knockout mice were generated.
- ✓ The decline in muscle and cardiac functions was comparable between mdx52 mice and mdx52/*Utrn*$^{+/-}$ mice.

Methods

Treadmill
- Running speed started at 6 cm/sec, then speed was increased 2 cm/sec every minute until exhaustion.

Grip strength
- Forelimb grip strength was measured 3 times for each mice.
- Maximum grip strength and force-decline rate* were calculated.

* (3rd measurement / 1st measurement) × 100 (%)


LE8707 (Panlab)


MK-380Si (Muromachi Kikai)

Rotarod
- The rotarod started at 4 rpm and accelerates to 40 rpm over 300 seconds.
- Latency to fall was determined (300 sec at maximum).


MK-670(Muromachi Kikai)

Echocardiography
- During the measurement, the heart rate was adjusted to approximately 500 bpm under isoflurane anesthesia.
- Data were obtained by long-axis B-mode, short axis M-mode, PW-doppler, and tissue doppler.


Vevo 2100
(FUJIFILM Visualsonics)

Aging effects of muscle and cardiac function in B-10 mdx mice

✓ **The decline in muscle function was detected by the grip test and treadmill test, but not by the rotarod test.**

Animals
C57BL/10 Jic (WT)
C57BL/10-mdx (B10-mdx)
Timepoint
1, 1.5, 2, 3, 9, 12 and 17 months of age

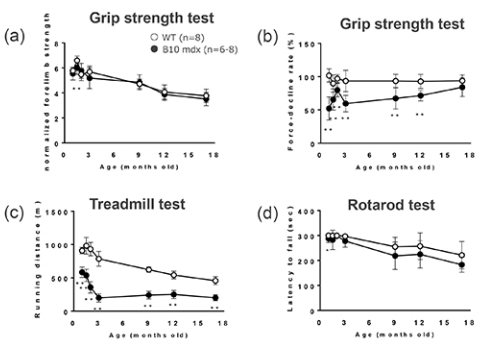

Fig.1 Time course of muscle functions in B10-mdx mice.
(a) Forelimb grip strength normalized by body weight, (b) Force-decline rate, (c) Running distance in treadmill test, (d) Latency to fall in rotarod test. Data are represented as the mean ± SD. * p<0.05, ** p<0.01 vs Wildtype group by Student's *t*-test or Wilcoxon test.

✓ **Systolic function mildly declined after 12 months of age.**
✓ **Prior to the decline in systolic function, diastolic dysfunction was observed.**

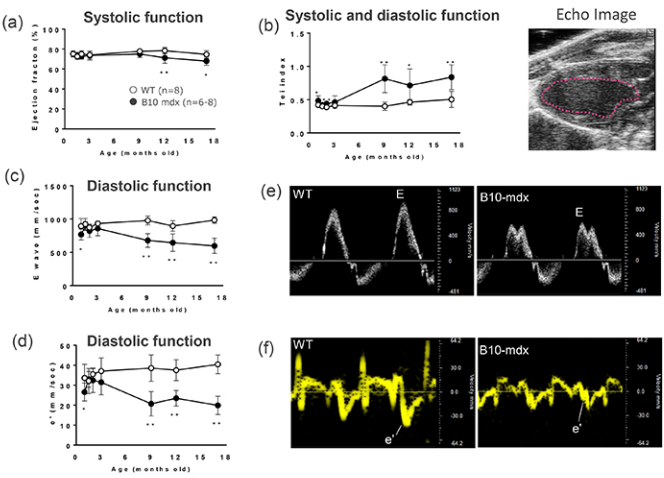

Fig.2 Time course of cardiac functions in B10-mdx mice measured by echocardiography.
(a) Ejection fraction, (b) Tei index (myocardial performance index), (c) E-wave (early diastolic wave), (d) e' (early diastolic velocity of the mitral annulus), (e) Representative PW-doppler images, (f) Representative tissue doppler image. Data are represented as the mean ± SD.
* p<0.05, ** p<0.01 vs wildtype group by Student's *t*-test or Wilcoxon test.

Comparison of muscle and cardiac function between mdx52 mice and mdx52/*Utrn*$^{+/-}$ mice

✓ **To expect more severe and early onset of the disease, we generated *Dmd* ex52 hemi knockout/*Utrn* hetero knockout mice.**

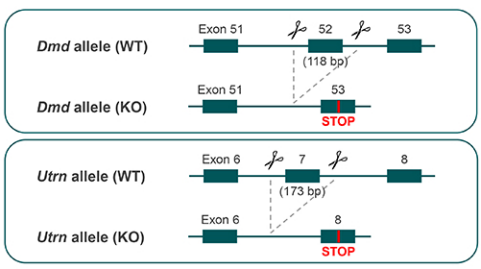

Fig.3 Generation of mdx52 mice and mdx52/Utrn$^{+/-}$ mice.
Mdx52 knockout (KO) mice were generated using CRISPR/Cas9 technology. Guide RNAs (gRNAs) were designed to flank *Dmd* exon 52, and the resulting frameshift mutation creates a stop codon at exon 53, leading to the knockout of the *Dmd* gene. *Utrn* KO mice were also generated using CRISPR/Cas9 technology. gRNAs were designed to flank *Utrn* exon 7, and the resulting frameshift mutation creates a stop codon at exon 8, leading to the knockout of the *Utrn* gene. Mdx52/*Utrn*$^{+/-}$ mice were obtained by crossing female heterozygous mdx52 mice with male heterozygous *Utrn* KO mice.

✓ **Muscle function and cardiac function at 13-week-old mdx52 and mdx52/*Utrn*$^{+/-}$ mice were evaluated.**
✓ **Both mdx52 and mdx52/*Utrn*$^{+/-}$ mice exhibited a decline in muscle function and diastolic function.**
✓ **No significant difference were noted between mdx52 and mdx52/*Utrn*$^{+/-}$ mice**

Animals
Wild type (WT)
Dmd ex52 hemi knockout (mdx52)
Dmd ex52 hemi knockout/*Utrn* hetero knockout (mdx52/*Utrn*$^{+/-}$)
Timepoint
3 months of age

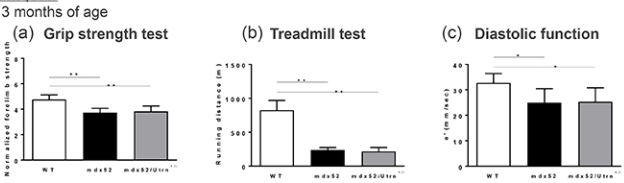

Fig.4 Comparison of muscle and cardiac functions between mdx52 mice and mdx52/Utrn$^{+/-}$ mice.
(a) Forelimb grip strength normalized by body weight, (b) Running distance in treadmill test, (c) e' (early diastolic velocity of the mitral annulus). Data are represented as the mean + SD.
* p<0.05, ** p<0.01 by Tukey.

We might generate *Dmd* ex52 hemi knockout/*Utrn* homo knockout mice to aim for a more severe disease model.

Conclusion

We evaluated the muscle and cardiac functions of B10-mdx mice over time and were able to detect functional decline. To expect more severe and early onset of the disease, we generated *Dmd* ex52 hemi knockout/*Utrn* hetero knockout mice and evaluated the function similarly. Unfortunately, the severity of functional decline in mdx52/Utrn$^{+/-}$ mice was comparable to that in mdx52 mice. We might generate *Dmd* ex52 hemi knockout/*Utrn* homo knockout mice to aim for a more severe disease model.

Reference

1) Kristy Swiderski, *et al.*, *Am J Physiol Cell Physiol*. 2021 Aug 1;321(2)
2) Gisela Gaina, *et al.*, *Exp Ther Med*. 2021 Jun;21(6):610
3) R M Grady, et al., *Cell*. 1997 Aug 22;90(4):729-38

COI disclosure information:
We have no financial relationship to disclose for our presentation contents.

If you are interested in the evaluation of cardiac function, muscle function, and dystrophin-utrophin double knockout, please contact the following.
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