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Study on the establishment of a system for measuring muscle strength of cisplatin-induced muscle atrophy model in mice

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Objective

Cisplatin is widely used in the treatment of various cancers, but it causes the atrophy of skeletal muscles. The atrophy might induce sarcopenia, which greatly reduce the quality of life in patients. Therefore, it is necessary to establish an evaluating test system of muscle weakness in muscle atrophy models.

Continuous periodic observations might be necessary to obtain precise information on the effects of chemicals on muscle function. In literature, cisplatin-induced muscle atrophy and improving effect of D-methionine in mice have been reported. Therefore, we examined the possibility of periodic quantifying the effects of D-methionine on cisplatin-induced muscle atrophy model by measuring muscle strength using 1300A, 3-in-1 Whole Animal System (Aurora scientific).

Summary in Japanese

抗がん剤の一種であるシスプラチンは，様々ながんの治療に広く使用されるが，重篤な副作用を伴うという問題点がある．その副作用の一つとして，骨格筋の萎縮が引き起こされることが，多数報告されている．筋委縮によるサルコペニアや，それに続く身体的フレイルは，患者のQOLを大きく低下させるため，薬の開発が必要である．また，薬の開発には筋委縮モデルにおける筋力低下を評価可能な試験系の確立が必要である．

本試験において，雄性C57BL/6J にシスプラチン (3 mg/kg/day, i.p.) を投与して筋委縮を惹起し，それに対して必須アミノ酸であるD-メチオニン (300 mg/kg/day, p.o.) を投与して効果を検証した．筋力はシスプラチンの初回投与より4日，7日および10日後に測定した．結果，作製したモデルの筋力は，正常マウスに比べ低下しており筋委縮が確認された．また，D-メチオニンが，筋委縮に伴う筋力低下を抑制することが確認された．この結果により，筋委縮モデルにおける筋力の経時的かつ定量的な測定を行う試験系を確立することができた．

Materials and Methods

● Animal

Male mice, C57BL/6J, 8-week-old

● Model preparation

Cisplatin was administrated by i.p. at a dose of 3 mg/kg for 9 days, once a day.

● D-methionine administration

D-methionine was administrated by p.o. at a dose of 300 mg/kg, once a day and started 3 days before the first cisplatin administration.

● Group Configuration

Group	Cisplatin Dose and Route	D-methionine Dose and Route	n
Normal	Saline, i.p.	Saline, p.o.	10
Control	3 mg/kg, i.p.	Saline, p.o.	10
D-methionine	3 mg/kg, i.p.	300 mg/kg, p.o.	10

● Muscle force measuring

In this study, the muscle force was measured by 1300A 3-in-1 Whole Animal System (Aurora Scientific).

The mice were anesthetized by isoflurane inhalation and placed in the thermostatically controlled table.

The foot firmly fixed to the footplate on the motor shaft. The muscle force values were obtained via electrical stimulation of the plantar flexor muscles through the tibial nerve and measured with the dynamometer. Measurements were conducted at intervals of 4, 7 and 10 days after cisplatin administration.

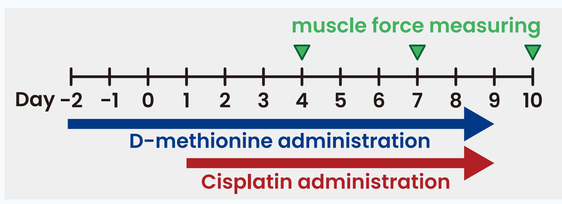
● Measurement condition

- Tetanus force: 100Hz, 0.35sec
- Force frequency: 20, 40, 60, 80, 100, 150 and 200 Hz, 0.35 sec, 60 sec interval
- Fatigue: 30 Hz, 0.35 sec, 2 sec interval, 120 sec duration (60 stimulations)

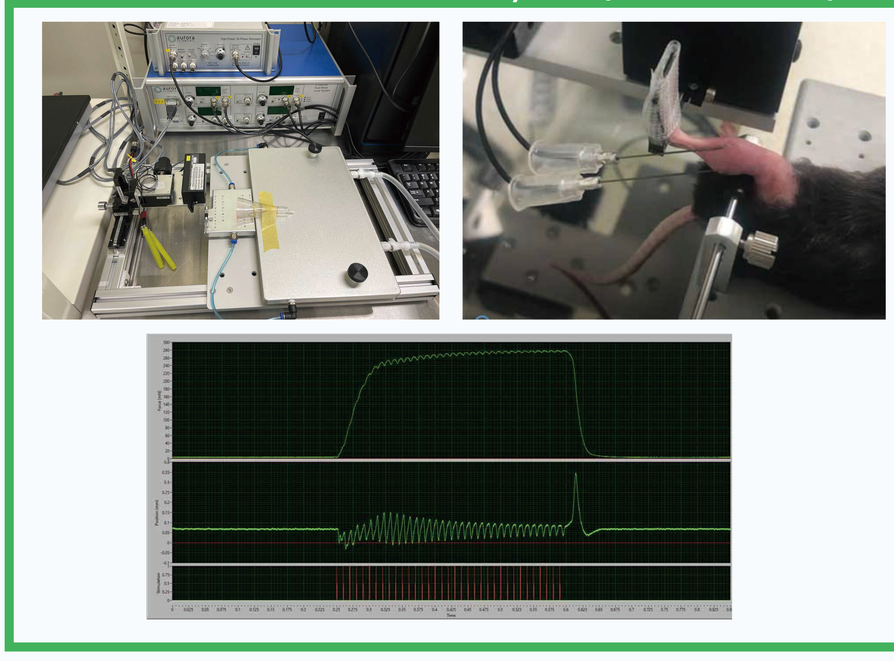
● Gastrocnemius muscle mass

Sampled gastrocnemius muscle and measured mass at days 10 after cisplatin administration.

● Schedule

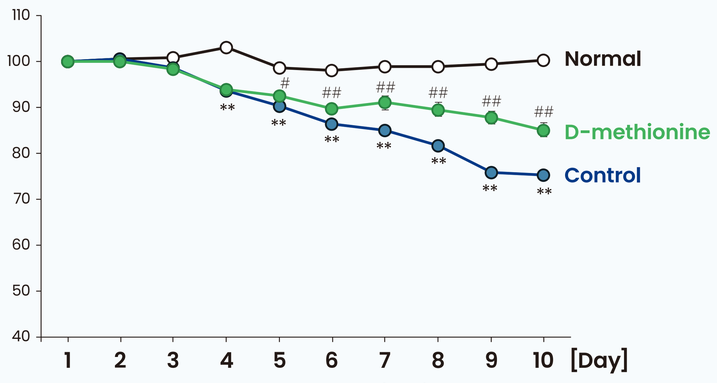


1300A 3-in-1 Whole Animal System (Aurora Scientific)



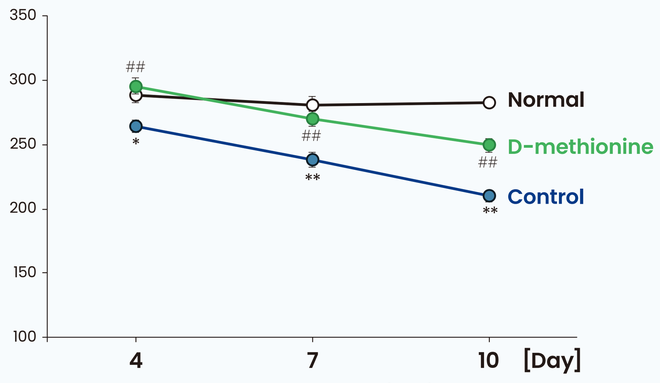
Results

Fig. A Body weight change rate (%)



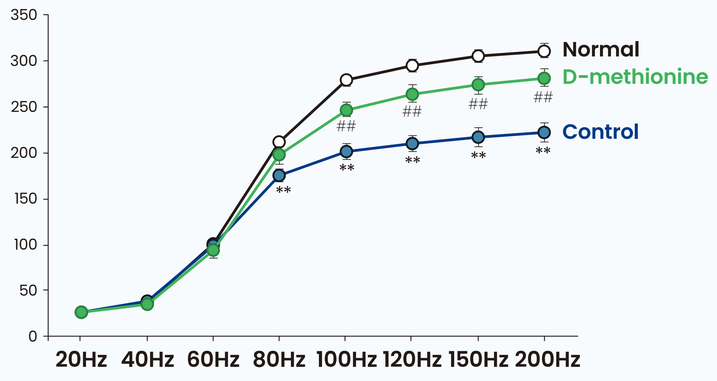
Each value represents the mean±S.E. (n=10)
**P<0.01 vs Normal (Student's *t*-test)
#P<0.05, ##P<0.01 vs Control (Student's *t*-test)

Fig. B Tetanus force (mN)



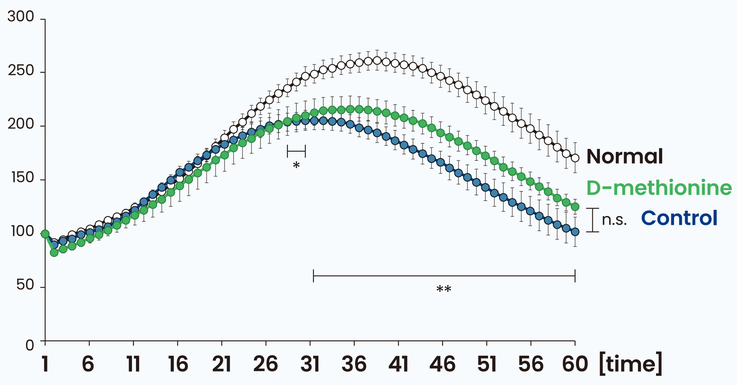
Each value represents the mean±S.E. (n=10)
*P<0.05, **P<0.01 vs Normal (Student's *t*-test)
##P<0.01 vs Control (Student's *t*-test)

Fig. C Force frequency-Day 10 (mN)



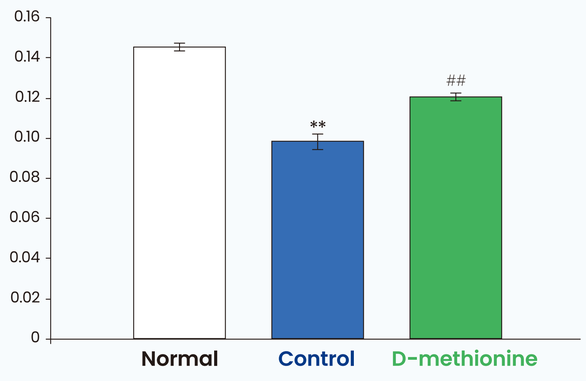
Each value represents the mean±S.E. (n=10)
**P<0.01 vs Normal (Two-way ANOVA, Student's *t*-test)
##P<0.01 vs Control (Two-way ANOVA, Student's *t*-test)

Fig. D Fatigue-Day 10 (% Initial Force)



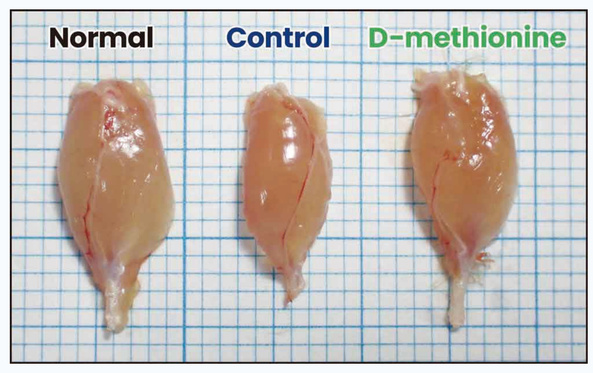
Each value represents the mean±S.E. (n=10)
*P<0.05, **P<0.01 vs Normal (Two-way repeated measures ANOVA, Student's *t*-test)
n.s. , No significant difference vs Control (Two-way repeated measures ANOVA)

Fig. E Gastrocnemius muscle mass (g)



Each value represents the mean±S.E. (n=10)
**P<0.01 vs Normal (Student's *t*-test)
##P<0.01 vs Control (Student's *t*-test)

Photo 1 Gross pathology of gastrocnemius muscle



Summary and Conclusion

- In control group, significant decrease in body weight after cisplatin administration was observed. In contrast, this decrease was significantly suppressed compared with control group in D-methionine-treated group [**Fig A**].
- Plantar flexion contractile force on day 4, 7 and 10 were significantly decreased in control group compared with normal group, and this decrease of muscle force was significantly suppressed compared with control group in D-methionine-treated group [**Fig B, C**].
- The fatigue data decline was faster in control group than normal group. While fatigue ability improved by approximately 20% compared to the control group after the 36th stimulus in D-methionine-treated group, D-methionine did not significantly affect fatigue ability [**Fig D**].
- Gastrocnemius muscle mass was significantly reduced in control group compared with normal group, and the reduction of the gastrocnemius muscle mass was significantly suppressed in D-methionine-treated group compared with control group [**Fig E, Photo 1**].
- From the above, the muscle strength of the cisplatin-induced muscle atrophy mice was lower than that of normal mice, and D-methionine improved muscle weakness.

In conclusion,

This measurement system was able to detect the decline in muscle force of muscle atrophy model with high sensitivity, and useful for evaluating changes in mouse muscle force periodically.

The model would be useful to evaluate potential efficacy of newly therapeutic chemicals on myofunction.