SciMedCentral

JSM Internal Medicine

Research Article

Appropriate Running Speed for Physical Rehabilitation in Brain-Injured Young Mice

Feifei Wang[1]*, Nagamasa Maeda[1,2], and Yusuke Sagara[1]

[1]*Center for Innovative and Translational Medicine, Kochi University Medical School, Japan*

[2]*Department of Obstetrics and Gynecology, Kochi University Medical School, Japan*

***Corresponding author**

Feifei Wang, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan, Tel: +81-88-880-2461; Email: f-wang@kochi-u.ac.jp

Submitted: 26 August 2016

Accepted: 18 November 2016

Published: 21 November 2016

Keywords

- Rehabilitation
- Hypoxic-ischemic brain injury
- Treadmill

Abstract

Physical exercise is beneficial for functional recovery after brain injury. For effective rehabilitation, the determination of appropriate running condition is an important challenge. In the present study, we examined the running parameter for treadmill exercise in a mouse model of neonatal hypoxic-ischemic brain injury. The mice performed exhaustive runs and incremental test to determine their critical speed (CS) and the velocity at the lactate threshold (vLT). The CS and vLT were significantly different between the normal mice and brain-injured mice. It was also found that the CS was not significantly different from vLT.

According to this study, CS and vLT could be valuable parameters for rehabilitation in a mouse model of neonatal hypoxic-ischemic brain injury.

ABBREVIATIONS

CS: Critical Speed; vLT: Velocity at the Lactate Threshold

INTRODUCTION

Regular aerobic exercise improves motor and cognitive symptoms associated with a variety of central nervous system disorders [1-4]. Previous studies have demonstrated that treadmill training can significantly reduce brain infarct volume and improve neurological outcomes in animal models of stroke [5]. Moreover, early exercise was found to decrease neuronal apoptosis in the cerebral cortex in rat stroke models [6].

However, there are only a few studies about the rehabilitation for animal models of neonatal hypoxic-ischemic brain injury. Since unsuitable exercise provides physiological stress to mice, it is important to determine the appropriate running speed for mice. Billat et al., have previously defined critical speed (CS) [7], which is a parameter of motor performance, and lactate threshold velocity (vLT) [8], which is an important factor in endurance events, in various strains of mice [9].

In the present study, we examined the running parameters for treadmill exercise in a mouse model of neonatal hypoxic-ischemic brain injury. We determined their CS and vLT as these parameters could be of assistance to various studies for the treatment of various brain disorders.

MATERIALS AND METHODS

Neonatal ischemia-reperfusion brain injury

All animals were used according to the Guidelines of the Institutional Animal Care and Use Committee of Kochi University. The C57BL/6N mice and NOD/SCID (NOD.CB17-Prkdcscid/J) mice were purchased from Charles River Laboratories Japan Inc. (Kanagawa, Japan). NOD/SCID mice were maintained in barriers and were fed sterile food and chlorinated sterile water. The modified Rice-Vannucci model [10,11] was used for neonatal ischemia-reperfusion brain injury as previously described [12]. Seven-day-old postnatal mice of both sexes (n = 107) were anesthetized with 2% isoflurane. The right common carotid artery was occluded with an aneurysm clip (Mizuho, Japan). The pups were placed in a hypoxia chamber held at 8% O_2 for 120 min. Reperfusion was achieved by unclamping the artery and exposing the pups to normoxic conditions. After the operation, they were returned to their dams.

Behavioral tests

The mice were evaluated behaviorally with an accelerating rotarod (Muromachi Kikai, Kyoto, Japan) and a hanging wire grip test (O'HARA & CO., LTD., Tokyo, Japan). Both the tests were performed at 3 weeks after the brain injury. The automated rotarod system was set to 4–40 rpm acceleration. The maximum time on the rod was 300 s. The latency to fall from the rod was recorded. Mice clinging to the rod and rotating were scored as a fall. In the hanging wire grip test, the latency to fall from the grip was recorded in each trial. Each mouse was pre-trained for 2 days before the tests. The analysis of each measure was based on the mean score of 3 trials.

Evaluation of CS

The evaluation of CS was started at 2 weeks post-injury using

a motor-driven treadmill with an electric stimulus at the rear of the belt (Figure 1A, MK-680S; Muromachi Kikai, Kyoto, Japan). The protocol was performed according to previous studies [9,13,14]. Briefly, a single trial per day consisted of a run at a constant speed (5–35 m/min). The time spent for running was recorded and limited to 45 min or exhaustion, as defined by a total number of 50 electric stimuli. The total running distance at a given speed and the time to cover the distance were used to calculate CS.

Incremental exercise test

The incremental exercise test was performed to determine vLT according to a previous study [9]. Three days after the evaluation of CS, the incremental test was performed that included a starting speed of 70% of their CS for 3 minutes. Then a 10% increased speed was applied every 3 min to reach the final speed of 130% of CS for 3 min. Blood lactate concentration was measured using a lactate pro-LT device (Arkray, Kyoto, Japan) from the tip of the tail. The vLT was defined as the speed at which an increase of >1 mM occurred.

Statistical analysis

Data are presented as means ± SD. Statistical significance was set at $P < 0.05$. Both the groups were compared using the one-way analysis of variance.

RESULTS AND DISCUSSION

In the rotarod test and hanging wire grip test, the brain-injured NOD/SCID mice performed significantly worse (INJURY: 150 ± 4.5s, 39 ± 2.1s, *p<0.05) compared to the uninjured NOD/SCID mice (NORMAL: 246 ± 3.7s, 97 ± 12.1s, *p<0.05) at 3 weeks after injury. The difference between normal group and injury group was larger in hanging wire grip test than the rotarod test (Figure 1). As shown in Figure (2), the CS was calculated from the slope of the regression line, while plotting the distance vs. the time to exhaustion for the three tests. In the mice, the blood lactate concentration of resting state was higher than that of humans (2.2–5.5 mM) (Figure 3).In the mouse strains, significant lower CS and vLT were observed in the brain-injured mice. Although the vLT was not significantly different between the strains, CS was much higher in NOD/SCID mouse than in B6 mouse for several conditions. There was no significant difference between CS and vLT (Table 1).

In the mouse model of neonatal hypoxic-ischemic brain injury, tissue damage was observed in the cerebral cortex, hippocampus, and striatum [4]. These areas are involved in motor control. The motor function of brain-injured mice was significantly worse compared to the uninjured mice. The B6 mice are widely used for animal studies, and the NOD/SCID mice are characterized by the absence of functional T cells and B cells and reduced macrophage and natural killer (NK) cell function. These mice are used for the investigation of human cell transplantation, cancer, graft-versus-host disease, and human immune system [15]. The exercise capacity of NOD/SCID mice has not been reported. Interestingly, in our study, CS was higher in NOD/SCID mice than in B6 mice for several conditions. The vLT was significantly lower in the brain-injured mice compared to the uninjured mice. A recent study showed that neural stem/progenitor cell transplantation combined with treadmill exercise exerted functional recovery in mice with chronic spinal cord injury [16]. The possible neuroprotective mechanisms of physical activity include the activation of neurogenesis, angiogenesis, and synaptic plasticity [17]. Although these parameters varied according to the conditions of the mice, the CS and vLT might be applied in rehabilitation research for brain-injured mice.

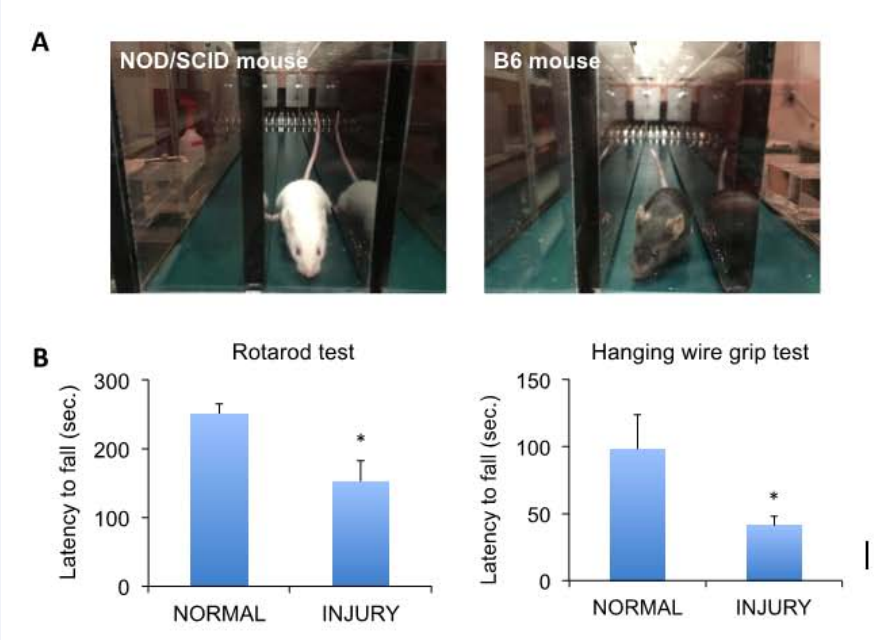

Figure 1 (A) Mice and treadmill apparatus used in this study.
(B) Behavioral tests for model mice. The brain-injured mice performed significantly worse at 3 weeks after injury compared to the uninjured mice.

Table 1: vLT and CS of B6 and NOD/SCID mice.

	B6 (Normal)			B6 (Injury)		
	3w old	**4w old**	**5w old**	**3w old**	**4w old**	**5w old**
CS (m/min.)	5.5 ± 1.2	16.1 ± 3.6	18.2 ± 2.33	5.1 ± 1.0	13.4 ± 1.3	15 ± 1.7
vLT (m/min.)	4.5 ± 0.7	14.0 ± 6.4	17.0 ± 1.73	3.8 ± 1.2	9.8 ± 4.7	14.0 ± 2.7
	B6 (Normal)			**B6 (Injury)**		
	3w old	**4w old**	**5w old**	**3w old**	**4w old**	**5w old**
CS (m/min.)	7.7 ± 1.1	16.5 ± 0.36	28.5 ± 3.0	4.1 ± 1.9	7.6 ± 3.1	14.0 ± 2.6
LT (m/min.)	6.7 ± 4.6	15.3 ± 9.4	20.5 ± 6.3	5.0 ± 6.0	11.5 ± 4.5	16.3 ± 8.4

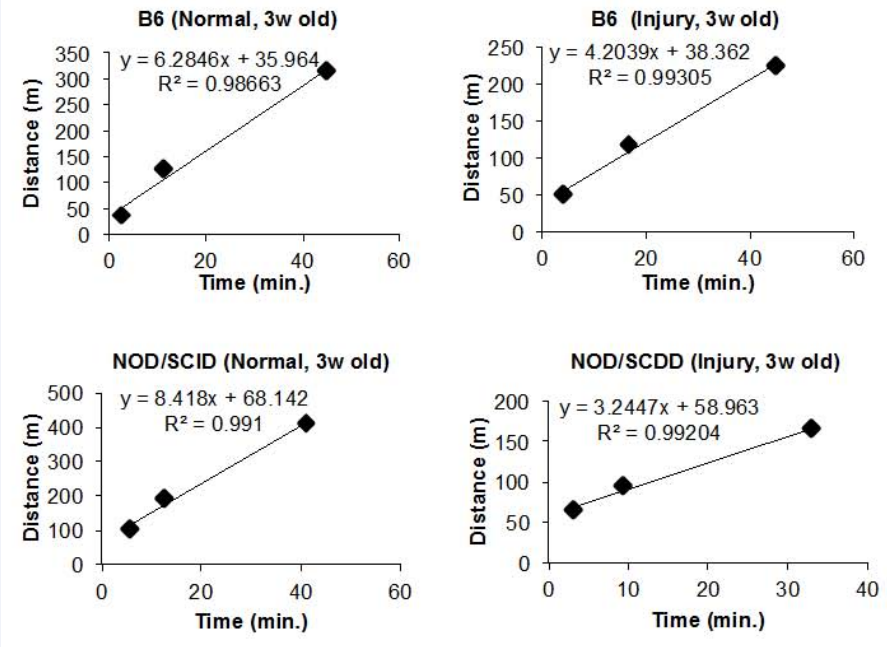

Figure 2 Determination of critical speed in B6 and NOD/SCID mice on the treadmill.

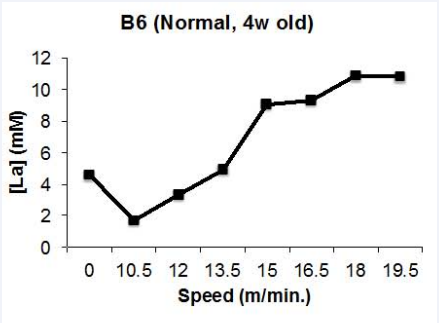

Figure 3 Determination of vLT in a 4-weeks-old B6 mouse on the treadmill.

CONCLUSION

In the present study, we examined CS and vLT in a mouse model of neonatal hypoxic-ischemic brain injury. CS and vLT were significantly lower in brain-injured mice compared to uninjured mice. Our findings could be of assistance to various studies for physical rehabilitation in brain-injured young mice.

ACKNOWLEDGEMENTS

The authors thank Ms. Kimiko Takaishi for assistance with animal surgery and exercise. This work was partly supported by Grant-in-Aid for Young Scientists (B), JSPS KAKENHI.

REFERENCES

1. Tajiri N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, et al. Exercise exerts neuroprotective effects on Parkinson's disease model of rats. Brain Res. 2010; 1310: 200-207.

2. Zhang QW, Deng XX, Sun X, Xu JX, Sun FY. Exercise promotes axon regeneration of newborn striatonigral and corticonigral projection neurons in rats after ischemic stroke. PLoS One. 2013; 8: e80139.

3. Chytrova G, Ying Z, Gomez-Pinilla F. Exercise normalizes levels of MAG and Nogo-A growth inhibitors after brain trauma. Eur J Neurosci. 2008; 27: 1-11.

4. Metrot J, Mottet D, Hauret I, van Dokkum L, Bonnin-Koang HY, Torre K, et al. Changes in bimanual coordination during the first 6 weeks after moderate hemipareticstroke. Neurorehabil Neural Repair. 2013; 27: 251-259.

5. Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schröck H, et

al. Mechanisms of stroke protection by physical activity. Ann Neurol. 2003; 54: 582-590.

6. Zhang P, Zhang Y, Zhang J, Wu Y, Jia J, Wu J, Hu Y. Early Exercise Protects against Cerebral Ischemic Injury through Inhibiting Neuron Apoptosis in Cortex in Rats. Int J Mol Sci. 2013; 14: 6074-6089.

7. Moritani T, Nagata A, deVries HA, Muro M. Critical power as a measure of physical work capacity and anaerobic threshold. Ergonomics. 1981; 24: 339-350.

8. Nagata A, Muro M, Moritani T, Yoshida T. Anaerobic threshold determination by blood lactate and myoelectric signals. Jpn J Physiol. 1981; 31: 585-597.

9. Billat VL, Mouisel E, Roblot N, Melki J. Inter- and intrastrain variation in mouse critical running speed. J Appl Physiol (1985). 2005; 1258-1263.

10. Levine S. Anoxic-ischemic encephalopathy in rats. Am J Pathol. 1960; 36: 1-17.

11. Rice 3rd JE, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol. 1981; 9: 131-141.

12. Wang F, Shen Y, Tsuru E, Yamashita T, Baba N, Tsuda M, et al. Syngeneic transplantation of newborn splenocytes in a murine model of neonatal ischemia-reperfusion brain injury. J Matern Fetal Neonatal Med. 2015; 28: 842-847.

13. Clingeleffer A, McNaughton LR, Davoren B. The use of critical power as a determinant for establishing the onset of blood lactate accumulation. Eur J Appl Physiol Occup Physiol. 1994; 68: 182-187.

14. di Prampero PE. The concept of critical velocity: a brief analysis. Eur J Appl Physiol Occup Physiol. 1999; 80: 162-164.

15. Gonzalez L, Strbo N, Podack ER. Humanized mice: novel model for studying mechanisms of human immune-based therapies. Immunol Res. 2013; 57: 326-334.

16. Tashiro S, Nishimura S, Iwai H, Sugai K, Zhang L, et al. Functional Recovery from Neural Stem/Progenitor Cell Transplantation Combined with Treadmill Training in Mice with Chronic Spinal Cord Injury. Sci Rep. 2016; 6: 30898.

17. Barber SE, Clegg AP, Young JB. Is there a role for physical activity in preventing cognitive decline in people with mild cognitive impairment? Age Ageing. 2012; 41: 5-8.

Cite this article

Wang F, Maeda N, Sagara Y (2016) Appropriate Running Speed for Physical Rehabilitation in Brain-Injured Young Mice. JSM Intern Med 1(1): 1001.