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Appropriate treadmill exercise improves survival after gut ischemia reperfusion in mice

Kazuya Higashizono [a, b], Kazuhiko Fukatsu [b, *], Ayako Watkins [b], Tomoki Watanabe [b], Midori Noguchi [b], Satoshi Murakoshi [b], Hiroshi Yasuhara [b], Yasuyuki Seto [a]

[a] Department of Gastrointestinal Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[b] Surgical Center, The University of Tokyo Hospital, Tokyo, Japan

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SUMMARY

Background & aims: It is unclear whether or not an appropriate prehabilitation protocol ameliorates surgical stress, ultimately improves survival after severe surgical insults.
Methods: Male C57BL/6J mice received treadmill exercise according to the following protocols. **Exp.1)** Sixty-min treadmill running at 20 m/min. The exercise sessions were 5 days/week for 2 weeks, 3 days/week for 2 weeks or 3 days/week for 3 weeks (n = 29). Sedentary mice (n = 6) did not perform treadmill exercise. After finishing each protocol, the exercise completion rate was evaluated. All mice which had completed the protocol (n = 24) underwent 45 min superior mesenteric artery occlusion and reperfusion (Gut I/R) and survival after gut I/R was observed. **Exp.2)** According to the results of Exp.1, the exercise frequency and duration were set at 3 days/week for 3 weeks. To determine the appropriate running speed, mice (n = 94) ran on the treadmill for 60 min at 12, 15 or 18 m/min, while sedentary mice (n = 25) did not. The exercise completion rate and survival were observed.
Results: **Exp.1)** The 3 days/week for 3 weeks exercise protocol showed the highest survival rate. However, the completion rate of each exercise group was only 50–60%. **Exp.2)** The completion rates at 15 m/min and 18 m/min were both approximately 90%. None of the 12 m/min group failed to complete the protocol. The 12 m/min

* Corresponding author: Kazuhiko Fukatsu, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail address: kazfukatsu@yahoo.co.jp (K. Fukatsu).

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and 15 m/min treadmill running groups showed equally improved survival as compared to the sedentary group ($p < 0.05$).
Conclusions: An appropriate exercise protocol improves survival after gut I/R in mice. Prehabilitation strengthens resistance to severe surgical insults.

1. Introduction

Exercise training before surgery, so-called prehabilitation, is expected to accelerate patient recovery through the maintenance of muscular strength and mass after surgery [1]. Because exercise training is known to modulate host responses to various insults, prehabilitation may also prevent organ dysfunction and poor outcomes following severe surgical stress.

In this study, we examined whether prehabilitation actually improves survival after severe gut ischemia reperfusion in mice. We also examined a number of exercise protocols to determine which would be the most appropriate for achieving optimal survival with high tolerability.

2. Material and methods

2.1. Animal models

This study conformed to the guidelines for the care and use of laboratory animals established by The University of Tokyo Hospital, and the protocols were approved by our animal care committee. Five-week-old male C57BL/6J mice were housed under a light/dark schedule of 12:12 h with *ad libitum* access to a standard diet (AIN-92M) and water.

2.2. Exercise completion criteria

Mice simultaneously exercised on a treadmill machine (Melquest, Toyama, Japan) with a rear shock grid (stimulatory shock of 0.3 mA) to maintain their running performance. The completion rate was evaluated based on drop-out criteria; Mice were judged to have dropped out when they touched the rear shock grid three or more times in a minute, or could no longer run.

2.2.1. Gut ischemia reperfusion

Animals were anesthetized with a subcutaneous injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). After anesthesia, the small intestine was exteriorized with a 1-cm midline laparotomy, and the superior mesenteric artery (SMA) was identified and occluded with a non-crushing microvascular clip for 45 min. The laparotomy incision was temporarily closed during SMA occlusion and then reopened to remove the clip with a 1.0-ml s.c. injection of saline solution just after reperfusion.

2.2.2. Statistical analysis

The log-rank test was used to calculate the exercise completion rate and survival duration. A value of p less than 0.05 was considered to indicate a statistically significant difference. The data are expressed as means ± SE. All statistical analyses were performed with JMP Pro 13 (SAS Institute Japan Ltd).

3. Experimental design

3.1. Experiment 1: Determination of appropriate running duration and frequency

Mice were familiarized with running on a treadmill at a speed of 12.0 m/min for 15 min on three days. Then, all mice subjected to the exercise protocols were randomized to 5 days/week for 2 weeks, 3 days/week for 2 weeks or 3 days/week for 3 weeks ($n = 29$). All mice in the exercise groups performed 60 min of running at a speed of 20 m/min for one day. The sedentary group did not run during this period. The exercise completion rate was observed after finishing the protocol. After treadmill running, all mice ($n = 16$) that completed the exercise protocol were used for the survival experiment. Survival was monitored for 48 h.

3.2. Experiment 2: Determination of appropriate running speed

According to the results of Exp.1, the training frequency and duration were set at 3 days/week for 3 weeks. Because exercise completion rates were very low in experiment 1, we sought a more appropriate running speed. After three days of warm-up (10.0 m/min for 15 min), the mice ($n = 94$) were randomized to 12, 15 or 18 m/min speed groups, while sedentary ($n = 25$) mice did not exercise. The exercise completion rate and survival after gut IR were observed.

4. Results

4.1. Experiment I: Determination of appropriate running duration and frequency

The completion rate at the end of the exercise period was approximately 60% in each group. There were no significant differences between any two of the groups (Fig. 1A). There were no significant differences in survival among any of the groups. The 3 days/week for 3 weeks exercise protocol apparently resulted in the highest survival time. Exercise for 5 days/week for 2 weeks failed, however, to improve survival as compared to that of the sedentary group (Fig. 1B).

4.2. Experiment II: determination of appropriate running speed

None of the 12 m/min group mice failed to complete the protocol. The 15 and 18 m/min groups also both showed good completion rates (Fig. 2A). All mice in the exercise groups showed better survival than the sedentary mice. Particularly, the 12 m/min and 15 m/min groups showed significantly improved survival as compared to the sedentary group (Fig. 2B).

5. Discussion

In the present study, we found that 12 m/min and 15 m/min treadmill running protocols improved survival after gut I/R and that no mice failed to complete the 12 m/min exercise protocol. To our best our knowledge, this is the first report clarifying that prehabilitation improves survival after severe gut I/R in mice. Because gut I/R is considered to be an important mechanism underlying organ dysfunction and death after severe surgical insults in clinical settings [2] appropriate prehabilitation with high tolerability might be a new strategy for preventing postoperative complications in addition to maintenance of muscle function. The present study also revealed that excessively demanding protocols may result in poorer outcomes. Therefore, we must be very cautious when designing prehabilitation protocols.

The mechanism underlying the improved survival is not clear from this study. Recently, muscle has attracted considerable attention as a regulator of physiological states through the

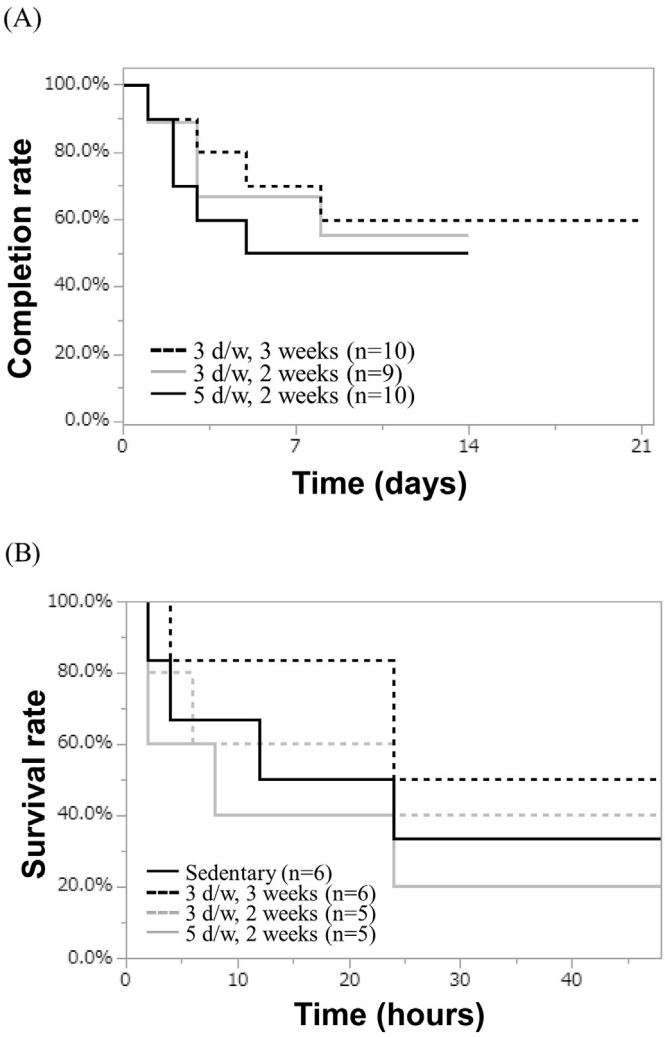

Fig. 1. (A) Exercise completion rate with 60 min of running at 20 m/min. (B) Survival after gut I/R with 60 min of running at 20 m/min.

secretion of myokines which are known to modulate inflammation [3]. It is possible that myokine levels during surgical stress were favorably changed. Alternatively, treadmill running may have caused minor gut ischemia repeatedly and thereby strengthened tolerance to severe ischemia. Indeed, this phenomenon is known as ischemia preconditioning [4]. Of course, our treadmill running protocol itself is not directly applicable to humans. However, the fact that an exercise protocol results in a high completion rate of exercise and good survival after gut I/R in mice suggests that an appropriate prehabilitation protocol may clinically contribute to the prevention of postoperative complications.

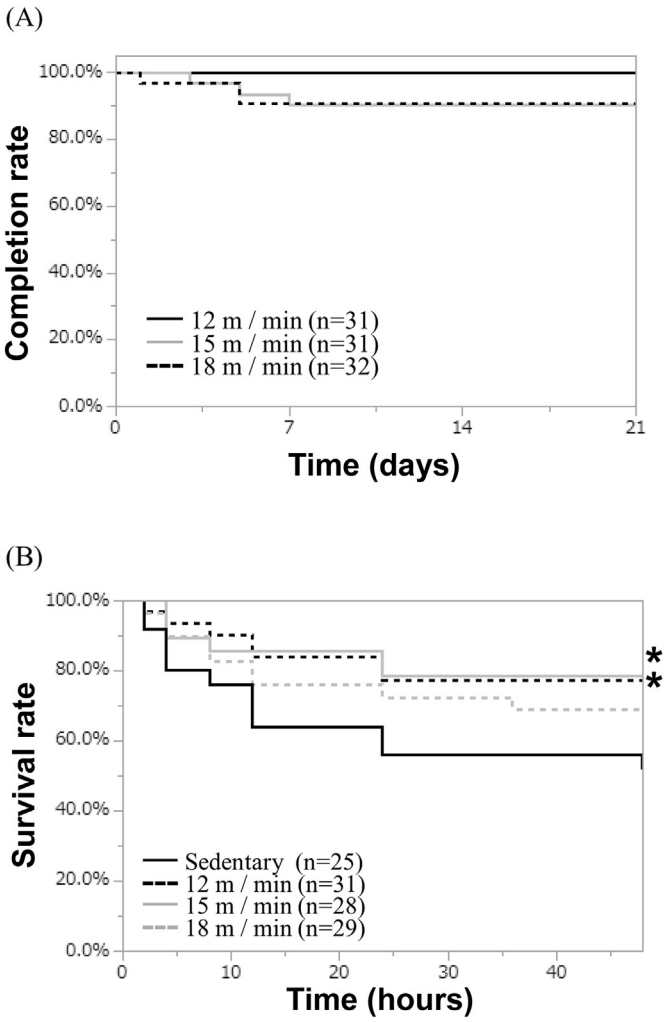

Fig. 2. (A) Exercise completion rate with 60 min of running at 3 days/week for 3 weeks. (B) Survival after gut I/R with 60 min of running at 3 days/week for 3 weeks. *P < 0.05 versus Sedentary group.

6. Conclusions

An appropriate exercise protocol resulted in a high completion rate of exercise and good survival after gut ischemia reperfusion in mice.

Statement of authorship

KF and SM designed the study. KH, AW, TW, and MN performed the experiments. KF, YS, and HY drafted and revised the article. All authors have approved the final article.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yclnex.2019.03.001.

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