The study on activation of ERK1/2 and Akt/mTOR signaling pathway in the soleus according to time point of exercise before or after sciatic nerve injury

Ji-eun Kim, Yeong-hyun Cho, Hyuk Sakong, Young-jun Park, Tae-beom Seo

Department of Kinesiology, College of Natural Science, Jeju National University, Jeju, Korea

1. Introduction

- Sciatic nerve injury (SNI) results in complex biological alterations including demyelination, axonal denervation and muscle atrophy, which are directly related to quality of life [1].
- Various exercise interventions have suggested positive therapeutic effect on nerve regeneration after SNI.
- Among various types of exercises, an aerobic exercise increases mitochondria activation and protein synthesis involved in axonal regrowth and muscle hypertrophy [2].
- It has been known that activation of Akt/mTOR signaling pathway regulates Schwann cell proliferation in the injured peripheral nerves as well as hypertrophy in the skeletal muscles [3].
- However, effect of time of application of exercise on activation of Akt/mTOR signaling pathway after SNI have not been studied in detail.
- Thus, the purpose of this study is to analyze whether treadmill exercise before or after or before and after SNI can increase expression levels of proteins associated with axonal growth and muscle hypertrophy.

2. Materials and Methods

2.1 Experimental animals

- Sprague–Dawley rats (6-wk-old male, body weight 190-200 g) were used in this experiment and divided into 5 groups
- Normal group (Norm, n=10), sedentary after SNI group (SAI, n=10), exercise before SNI group (EBI, n=10), exercise after SNI group (EAI n=10), and exercise before and after SNI group (EBAI, n=10).

2.2 Treadmill exercise protocol

- The experimental rats were adapted to treadmill exercise for a week before starting the experiment.
- The animals had rest for 2 days after sciatic nerve injury, and then animals in treadmill exercise group ran on treadmill device at a speed of 8 m/min for 20 min once a day according to exercise duration.

2.3 Sciatic nerve injury

- The rats were anesthetized with using an animal inhalation necrosis control.
- The left sciatic nerve was crushed with a pair of forceps for 1 min and 30 sec at interval [4].
- After surgery, anesthetized animals were then placed on a heating pad maintained at 37°C, and then they were put in their cages for resting.

2.4 Western blot

- The dissected sciatic nerves were rinsed with PBS, and lysed in Triton lysis buffer.
- Being denatured proteins were separated on SDS-polyacrylamide gel and then transferred onto PVDF membrane on ice at 200 mA for 2 hours.
- Protein (20μg) was used for Western analysis using anti-GAP-43 antibody (1:1000, rabbit monoclonal, Santa Cruz), anti-Erk1/2 antibody (1:1000, rabbit polyclonal, Cell Signaling), anti-p-Akt antibody (1:1000, rabbit polyclonal, Cell Signaling), anti-p-mTOR antibody (1:1000, rabbit polyclonal, Cell Signaling), anti-4E-BP1 antibody (1:1000, rabbit polyclonal, Cell Signaling).

2.5 Statistical analysis

- Statistical analysis was performed using one-way ANOVA followed by Duncan *post hoc* test. The level of significance accepted was set at $p < 0.05$.

3. Results

3.1 Regular exercise performed pre and post-injury upregulated p-ERK1/2 levels in ipsilateral soleus.

- It has been known that p-Erk1/2 was activated by SNI, which is representing enhancement of Schwann cell proliferation
- EBI and EBAI group significantly increased p-Erk1/2 induction in the ipsilateral soleus compared to other groups

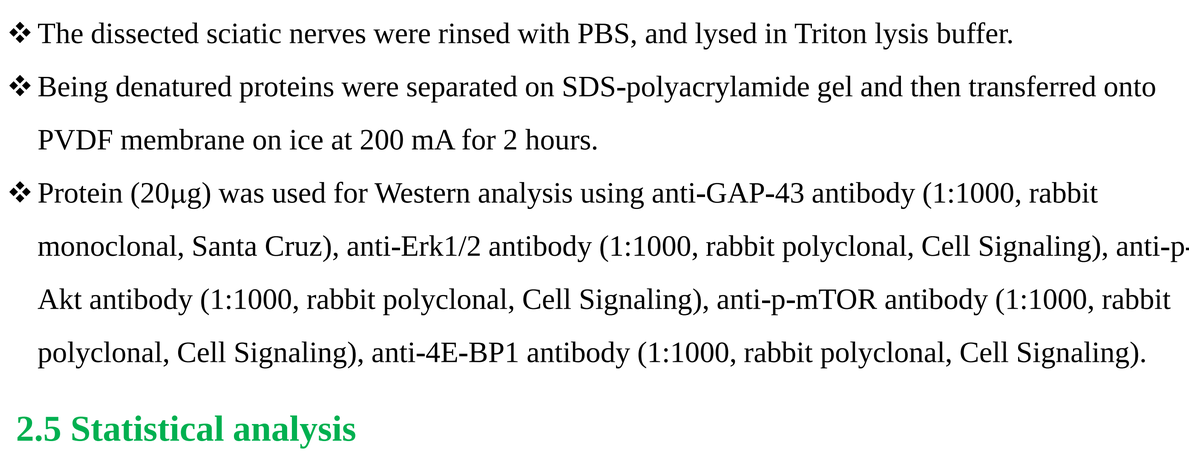

Figure 2. Differences in expression levels of p-Erk1/2 according to time point of exercise. GAPDH was detected as an internal loading control. $^{**}P<0.01$, $^{***}P<0.001$ vs. the SAI group. Norm, normal group; SAI, sedentary after injury; EBI, exercise before injury; EAI, exercise before injury; EBAI, exercise before and after injury.

3.2 Regular exercise performed pre and post-injury upregulated p-Akt levels in ipsilateral soleus.

- It has been known that p-Akt is closely associated with hypertrophy of skeletal muscle.
- EBAI group further activated p-Akt expression in the ipsilateral soleus than those in other group.

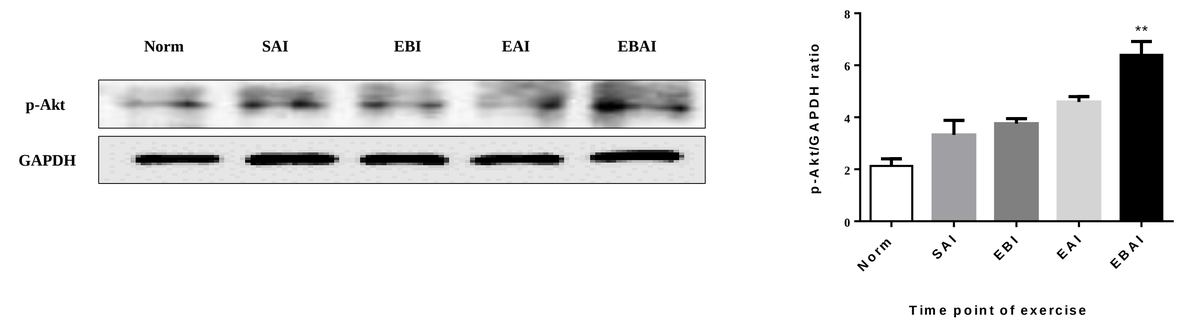

Figure 3. Differences in expression levels of p-Akt according to time point of exercise. GAPDH was detected as an internal loading control. $^{**}P<0.01$ vs. the SAI group. Norm, normal group; SAI, sedentary after injury; EBI, exercise before injury; EAI, exercise before injury; EBAI, exercise before and after injury.

3.3 Regular exercise performed pre and post-injury upregulated p-mTOR levels in ipsilateral soleus.

- It has been known that p-mTOR is involved in skeletal muscle hypertrophy
- EBI and EAI group significantly increased p-mTOR expression level in ipsilateral soleus compare to other group.
- EBAI group is not significantly elevated as much as EAI group because sustained aerobic exercise may inhibit mTOR by inducing adenosine monophosphate-activated protein kinase (AMPK) activity that promotes oxidative metabolism [5]

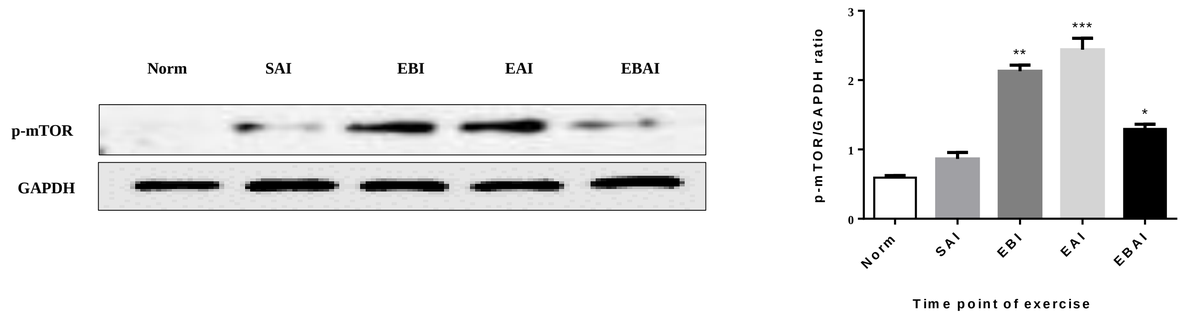

Figure 4. . Differences in expression levels of mTOR according to time point of exercise. GAPDH was detected as an internal loading control. $^{**}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. the SAI group. Norm, normal group; SAI, sedentary after injury; EBI, exercise before injury; EAI, exercise before injury; EBAI, exercise before and after injury.

3.4 Regular exercise performed pre and post-injury upregulated p-4E-BP1 levels in ipsilateral soleus.

- 4E-BP1 is a downstream molecule of mTOR and leads to protein synthesis in skeletal muscle
- EAI and EBAI significantly increased p-4E-BP1 levels in the ipsilateral soleus compared to other group.

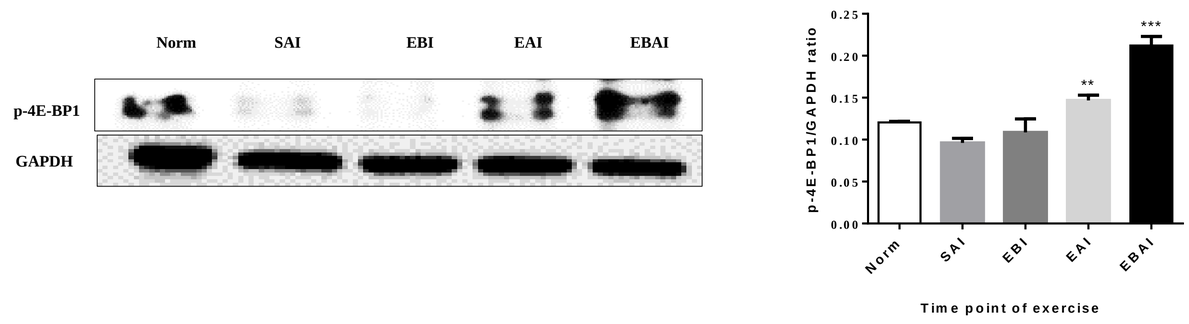

Figure 4. . Differences in expression levels of p-4E-BP1 according to time point of exercise. GAPDH was detected as an internal loading control. $^{**}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. the SAI group. Norm, normal group; SAI, sedentary after injury; EBI, exercise before injury; EAI, exercise before injury; EBAI, exercise before and after injury.

4.Conclusion

Our findings suggested new evidences that exercise performed pre and post-injury might activate Erk1/2 and Akt/mTOR signaling pathway in the ipsilateral soleus, representing increase of axonal regrowth and muscle hypertrophy.

References

1. Menorca et al., Hand Clin, 29:317-330, 2013.
2. Konopka et al., J Gerontology, 65A:1201-1207, 2010.
3. Maltzahn et al., J Nat Cell Biol, 2012.
4. Seo et al., J Neurotrauma, 26(10):1733-1744, 2009.
5. Woo et al., J Phys Ther Sci, 28(4):1260-1265, 2016.