Rapamycin Promotes Autophagy and Reduces Neural Tissue Damage after Spinal Cord Injury in Mice

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INTRODUCTION

Autophagy is a bulk degradation of subcellular constituents and is activated in several neurodegenerative conditions. Autophagy regulates the mechanism of cell death including apoptosis and autophagic cell death. We previously reported that autophagy increased after spinal cord injury (SCI) [1]. Rapamycin is known to activate autophagy by inhibiting the mammalian target of rapamycin (mTOR) [2]. Rapamycin promotes autophagy and reduces neural tissue damage after traumatic brain injury [3]. There has been no study to investigate the effect of rapamycin in SCI.

In the present study, we examined whether rapamycin promotes autophagy and reduces neural tissue damage after SCI.

MATERIALS AND METHODS

Animals Adult female C57BL/6J mice between 8 and 10 weeks of age were used.

Surgical procedures The T10 vertebra was laminectomized to expose the dura matter. SCI was induced with a modified NYU impactor.

Rapamycin injection Rapamycin (CALBIOCHEM) was dissolved in DMSO and injected intraperitoneally at a 1 mg/kg dose. Drug or vehicle injections were given at 4 hours after SCI.

Behavioral analysis Motor function of the hindlimbs was evaluated by the locomotor rating test using the Basso mouse scale (BMS) for 6 weeks after SCI.

Tissue preparations At 3 days and 42 days after SCI, mice were killed. Spinal cords were fixed with 4% paraformaldehyde and embedded in paraffin. Serial transverse sections at 250 µm intervals around the lesion epicenter were mounted on slides.

Counting of LC3-positive cells For detection of autophagy, the sections at 3 days after SCI were incubated with rabbit anti-LC3 antibody (1:100; MBL) and visualized by Alexa Fluor 594 goat anti-rabbit IgG (1:500; Molecular Probes). The total number of LC3-positive cells in 5 sections around the lesion epicenter was counted.

White matter staining To analyze areas of spared white matter, the sections from the epicenter to 1000 µm caudal side at 42 days were stained with Luxol Fast Blue. The images of the sections were captured by a digital photographic camera. White matter areas were measured by ImageJ 1.42q software.

Counting of NeuN-positive cells To investigate neuronal loss, the sections at 42 days were incubated with mouse anti-neuronal-specific-nuclear protein (NeuN) (1:100; Chemicon) and visualized by Alexa Fluor 488 goat anti-mouse IgG (1:500; Molecular Probes). The number of NeuN-positive cells around the lesion epicenter was counted.

RESULTS

Behavioral analysis From 14 days, rapamycin-treated mice showed higher BMS score than controls. There were significant differences between rapamycin-treated mice and controls from 28 days to 42 days (Fig. 1).

COUNTING OF LC3-POSITIVE CELLS

The number of LC3-positive cells in rapamycin-treated mice was significantly higher than that in controls (Fig. 2).

DISCUSSION

In the present study, the expression of LC3 in rapamycin-treated mice was significantly higher than that in controls. This result showed that rapamycin promoted activation of autophagy after SCI.

Rapamycin-treated mice showed better improvement of hindlimbs motion after SCI. The areas of white matter and the number of neurons in rapamycin-treated mice were preserved compared to controls. Rapamycin is considered to have a neuroprotective function. However, the relation of autophagy to neuroprotection is still unclear from the present study.

CONCLUSION

Rapamycin promoted autophagy and improved motor function and reduced neural tissue damage after SCI.

REFERENCES


Fig.1. BMS score (n = 5 per group, *p < 0.05). Error bars indicate SD.

Fig.2. Immunohistochemical staining of LC3 in control (A) and rapamycin-treated mouse (B) at 3 days. Scale bar indicates 100 µm. The graph (C) shows the number of LC3-positive cells (n = 4, *p < 0.05). Error bars indicate SD.

Fig.3. White matter staining with Luxol Fast Blue in control (A) and rapamycin-treated mouse (B) at 42 days. The areas enclosed by a yellow dotted line indicate spared white matters. Scale bar indicates 500 µm. The graph (C) shows the spared white matter area from the lesion epicenter to 1000 µm caudal side (n = 3 per group). Error bars indicate SEM.

Fig.4. Immunohistochemical staining of NeuN in control (A) and rapamycin-treated mouse (B) at 42 days. Scale bar indicates 100 µm. The graph (C) shows the number of NeuN-positive cells (n = 3, *p < 0.05). Error bars indicate SD.