INTRODUCTION
Autophagy is a bulk degradation of subcellular constituents and is activated in several neurodegenerative conditions. Autophagy regulates apoptosis and autophagic cell death. We previously reported that activity of autophagy increased after spinal cord injury (SCI) [1]. Rapamycin activates autophagy by inhibiting the mammalian target of rapamycin (mTOR) [2]. Rapamycin administration 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.

The purpose of the present study was to examine whether rapamycin promotes activity of autophagy and reduce neural tissue damage and locomotor impairment after SCI.

MATERIALS AND METHODS
Animals Adult female C57BL/6J mice (8-10 weeks of age) were used.
Surgical procedures The T10 vertebra was laminectomized to expose the dura mater. SCI was induced using a modified NYU impactor.
Rapamycin injection Rapamycin (1mg/kg) or vehicle was injected intraperitoneally at 4 hours after SCI.
Behavioral analysis Locomotor function of the hindlimbs was evaluated using the Basso mouse scale (BMS) for 6 weeks after SCI.

Counting of LC3- and Beclin 1-positive cells To examine the activity of autophagy, immunohistochemical staining of LC3 (marker of autophagy) and Beclin 1 (promoter of autophagy) was performed at 3 days. The numbers of LC3- and Beclin 1-positive cells were counted using 5 serial transverse sections at 250 µm interval around epicenter.

Western blot The expressions of LC3 and Beclin 1 after SCI were analyzed in Western blot at 3 days after SCI. The band densities were determined using ImageJ 1.42q software.

White matter staining To analyze areas of spared white matter, luxol fast blue staining was performed using the serial sections from epicenter to 1000 µm rostral and caudal side at 42 days.

Counting of NeuN-positive cells To investigate neuronal loss, immunohistochemical staining of NeuN was performed at 42 days. The number of NeuN-positive cells was counted.

RESULTS
Behavioral analysis

Fig. 1. BMS score. Rapamycin-treated mice had significantly higher BMS score than vehicle-treated mice from 28 days to 42 days (n = 5 per group, *p < 0.05).

Counting of LC3- and Beclin 1-positive cells

Fig. 2. Immunohistochemical staining of LC3 (A, B) and Beclin 1 (C, D) at 3 days. Scale bar: 100 µm. (E) The number of LC3-positive cells in rapamycin-treated mice was significantly higher than that in vehicle-treated mice. (F) The number of Beclin 1-positive cells was higher but not significant in rapamycin-treated mice (n = 4 per group, *p < 0.05).

DISCUSSION
In the present study, rapamycin-treated mice showed significantly better improvement of locomotor function after SCI. The areas of white matter and the number of neurons in rapamycin-treated mice were preserved compared to vehicle-treated mice. Thus, rapamycin is considered to have a neuroprotective function in SCI.

Rapamycin induced significant increase of the LC3 expression, but not Beclin 1 after SCI. Previous studies showed that rapamycin inhibits mTOR and then activates Atg1 kinase complex to regulate autophagy [4]. Our results demonstrated that rapamycin promoted autophagy in different mechanism from Beclin 1 after SCI.

CONCLUSION
Rapamycin promoted autophagy and reduced locomotor impairment and neural tissue damage after SCI.

REFERENCES