Rapamycin Suppresses Astrocytic and Microglial Activation and Reduced Development of Neuropathic Pain after Spinal Cord Injury in Mice.

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Disclosures:

Introduction:
The mammalian target of rapamycin (mTOR) signaling pathway plays an important role in multiple cellular functions. Many previous studies have shown that mTOR regulates both neuroprotective and neuroregenerative functions in trauma and various diseases in the central nervous system [1]. We recently reported that administration of mTOR inhibitor rapamycin reduces neural tissue damage and locomotor impairment after spinal cord injury (SCI) in mice [2].

Neuropathic pain conditions such as tactile and thermal allodynia are frequent secondary outcomes of SCI. The treatment to recover after SCI, the Basso mouse scale (BMS) and BMS subscore was evaluated for 42 days.

Rapamycin injection: Rapamycin (1mg/kg) or vehicle was injected intraperitoneally at 4 hours after SCI.

Locomotor function: To confirm the effect of rapamycin administration on locomotor recovery after SCI, the Basso mouse scale (BMS) and BMS subscore was evaluated for 42 days.

Mechanical allodynia: To evaluate mechanical allodynia in the hind paw, withdrawal threshold was measured using a von Frey filament (0.04-4g) applied to the plantar surface. A modification of the “up-down” method was used to determine the value at which paw withdrawal occurred 50% of the time [4]. Assessments of mechanical allodynia were evaluated every 7 days until 42 days after SCI.

Thermal allodynia: To evaluate thermal allodynia in the hind paw, withdrawal latency was measured using an infrared heat stimulus. An automatic plantar test instrument was used based on Hargreaves’ method [5].

Assessments of thermal allodynia were evaluated every 7 days until 42 days after SCI.

Immunohistochemistry: To examine the activation of glial cells in the lumbar spinal cord that indicates degree of allodynia, immunohistochemical stainings of Iba-1 for microglia and GFAP for astrocyte were performed using transverse sections of the lumbar spinal cord (L2/3) at 42 days. To quantify the activations of microglia and astrocyte, immunodensities of Iba-1- and GFAP-stained areas in the dorsal horn were measured using ImageJ 1.46r software. The immunodensities were compared among rapamycin-treated group, vehicle-treated group and sham group.

Results:
Locomotor function: Rapamycin-treated mice had significantly higher BMS score from 14 days and BMS subscore from 21 days than vehicle-treated mice. (Fig.1 A, B)

Mechanical allodynia: The withdrawal threshold was decreased from 14 days in vehicle-treated mice and 21 days in rapamycintreated mice after SCI. The withdrawal threshold in rapamycin-treated mice was consistently higher than vehicle-treated mice from 14 to 42 days. Mechanical allodynia in the rapamycin-treated mice significantly improved from 35 days compared to vehicle-treated mice (Fig. 2 A).

Thermal allodynia: The withdrawal latency were decreased from 7 days after SCI in both group. The withdrawal latency in rapamycin-treated mice significantly improved from 21 days compared to vehicle-treated mice (Fig. 2 B).

Iba-1 stainings: In representative pictures showed the number of Iba-1-stained microglia was obviously less in rapamycin-treated mice compared to vehicle-treated mice (Fig. 3 A, B). Immunodensity of Iba-1-stained area in the dorsal horn was significantly lower in rapamycin-treated mice than in vehicle-treated mice (Fig. 3 C).

GFAP stainings: In representative pictures showed the number of GFAP-stained astrocytes was less in rapamycin-treated mice compared to vehicle-treated mice (Fig. 4 A, B). Immunodensity of GFAP-stained area in the dorsal horn was relatively lower in rapamycin-treated mice than vehicle-treated mice (Fig. 4 C).

Discussion:
In the present study, the administration of rapamycin significantly improved not only locomotor function but also mechanical and thermal allodynia in the hind paw after SCI. Additionally, the analyses of Iba-1- and GFAP-stained areas confirmed that glial overactivation in the lumbar spinal cord actually suppressed in rapamycin-treated mice compared to vehicle-treated mice. Our results first demonstrated that treatment of rapamycin suppressed the activation of glial cells in the lumbar spinal cord and attenuated neuropathic pain after SCI. Our previous study demonstrated administration of rapamycin in acute phase of SCI significantly reduced secondly damage in the injured spinal cord [2]. The neuroprotective effect of rapamycin that reduces neural tissue damage may contribute to attenuation of neuropathic pain after SCI. Therefore, rapamycin treatment can be a novel therapeutic strategy to improve neuropathic pain following SCI.

Significance:
Rapamycin significantly improved not only locomotor function but also mechanical and thermal allodynia after SCI. The present study provided the first evidence that the treatment of rapamycin has a significant therapeutic effect to reduce neuropathic pain after SCI.

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References:
Fig. 2. Mechanical and thermal alldynia for 42 days after SCI. (A) In the assessment of mechanical alldynia, rapamycin-treated mice were significantly improved from 35 days. (B) In the assessment of thermal alldynia, rapamycin-treated mice were significantly improved from 21 days. (Rapamycin n=5, Vehicle n=7. *p<0.05)

Fig. 3. (A, B) Immunohistochemical staining for Iba-1 of lumbar spinal cord at 42 days. (Scale bar: 100μm) (C) Density of dorsal horn in rapamycin-treated mice was significantly lower than that in vehicle-treated mice. (Sham n=3, Vehicle n=7, Rapamycin n=5. *p<0.05, **p<0.01)
Fig. 4. (A, B) Immunohistochemical staining for GFAP of lumbar spinal cord at 42 days. (Scale bar: 100μm) (C) Density of dorsal horn in rapamycin-treated mice was relatively lower than that in vehicle-treated mice. (Sham n=3, Vehicle n=7, Rapamycin n=5)