ANTIOXIDANT EFFECT OF HEME OXYGENASE-1 PRESERVES SPINAL FUNCTION AFTER SPINAL CORD INJURY IN BACH1-DEFICIENT MICE

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Introduction
Oxidative stress, which is one of the mechanisms of secondary injury after spinal cord injury, leads to neural dysfunction and death. Among the consequences of oxidative stress, heme oxygenase-1 (HO-1) expression was observed in injured spinal cords. HO-1 was induced in microglia and macrophages from 24 hours to at least 42 days after injury. HO-1, which is a 32 kDa member of the heat shock protein family, catalyzes heme to iron, carbon monoxide, and biliverdin, that is rapidly reduced to bilirubin. Carbon monoxide, biliverdin, and bilirubin have antioxidant and anti-inflammatory activities. So, HO-1 expression protects cells from various insults including oxidative stress. Bach1 is a critical physiological repressor of HO-1. Bach-deficient (Bach1/-) mice express a high level of HO-1 at the mRNA and protein levels in various organs. Therefore, we hypothesized that the antioxidant effect of high HO-1 expression may preserve spinal function after spinal cord injury in Bach1/- mice.

Methods
Adult C57BL/6 male mice and homozygous Bach1/- C57BL/6 male mice (8-9 weeks old, weighing 20-23g) were used in this study (n=11 in each group). T11 laminectomy was performed, and spinal cord injury was made at the level of T11-12 by compressing the cord laterally from both sides with No.5 Dumont forceps for 10 seconds. The forceps were made at the level of T11-12 by compressing the cord laterally from both sides with No.5 Dumont forceps for 10 seconds. Bach1/- mice were prepared for the experiment one day in advance. The control group was the wild type mice (n=10 in each group).

Results
The BBB score gradually improved from day 1 (wild type, 11.4±5.2) to day 14 (Bach1/- mice, 78.7±17.6% of control) in all mice (wild type, 16.3±4.8; Bach1/- mice, 17.8±3.5) (Fig.3A). The BBB score of Bach1/- mice tended to be higher than that of wild type mice throughout the observation period, although the differences were not significant at any time point. On electrophysiological assessment, in the preinjury state there was no significant difference in the peak-to-peak amplitude of Br(E)-MsEP between the wild type (21.9±2.2mV) and Bach1/- mice (22.2±1.8mV). After spinal cord injury, the amplitude gradually recovered from day 1 (wild type, 26.1±6.8% of respective control; Bach1/- mice, 43.5±14.2% of respective control) to day 14 (wild type, 50.2±16.3% of control; Bach1/- mice, 78.7±17.6% of control) in all mice (Fig.3B). The peak-to-peak amplitude was significantly larger in Bach1/- mice than in wild type mice throughout the observation period (p<0.05 at each time point).

Discussion / Conclusions
In Bach1/- mice, HO-1 is expressed constitutively at high levels under normal physiological conditions. So, HO-1 inhibits oxidative stress just after spinal cord injury, and the peak-to-peak amplitude was significantly larger in Bach1/- mice than in wild type mice 1 day after injury. But, in wild type mice, HO-1 was induced in microglia and macrophages from 24 hours to at least 42 days after injury. So, there was no significant difference in the degree of recovery of the amplitude in either group on growth curve analysis. The results of this electrophysiological study suggest that high HO-1 expression inhibits oxidative stress and preserves spinal function in the early stage after spinal cord injury. Treatment that induces HO-1 expression at the early stage of spinal cord injury may preserve the functional outcome after the injury.

References

Fig. 1 Pathophysiology of secondary injury in the injured spinal cord.

Fig. 2 A, Spinal cord injury was made at the level of T11-12 by compressing the cord laterally from both sides with forceps with a 0.5mm spacer for 10 seconds. B, MEPs following transcranial electrical stimulation, which consisted of square constant current pulses, were recorded from both hamstring muscles [Br(E)-MsEP].

Fig. 3 A, BBB locomotor rating scale. The BBB score of Bach1/- mice tended to be higher than that of wild type mice throughout the observation period, although the differences were not significant at any time point. B, Motor evoked potentials (amplitude/control). The peak-to-peak amplitude was significantly larger in Bach1/- mice than in wild type mice throughout the observation period (p<0.05 at each time point).