Heme Oxygenase-1 Modulates the Secondary Injury Process after Spinal Cord Injury in Bach1 Deficient Mice

Yamada, K; Tanaka, N; Nakanishi, K; Sasaki, H; Kamei, N; Hamasaki, Ishikawa, Y; Yamamoto, R; Nakamae, T; Izumi, B; Ochi, M
Department of Orthopaedic Surgery, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan
yamada.kiyo@leaf.ocn.ne.jp

Introduction
Oxidative stress contributes to secondary injury after spinal cord injury. The expression of heme oxygenase-1 (HO-1), which protects cells from various insults including oxidative stress, is upregulated in injured spinal cords. Mice deficient in Bach1 (Bach1−/−), a transcriptional repressor of the heme oxygenase-1 and beta-globin genes, express high levels of HO-1 mRNA and protein in various organs. We hypothesized that HO-1 modulates the secondary injury process after spinal cord injury in Bach1−/− mice.

Methods
Experimental model: 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 modified with a spacer so that a 0.5mm space remained at maximal closure (Fig.1A).

Functional Assessment and Electrophysiological Recording: At 1, 2, 3, 5, 7, and 14 days after injury, recovery of hindlimb motor function was assessed using the BBB Locomotor Rating Scale, and signal conduction in the motor pathway was assessed by myoelectric motor-activated potentials (MEPs). MEPs following transcranial electrical stimulation, which consisted of square constant current pulses, were recorded from both hamstring muscles and triceps muscles as a transaction control. Expression of HO-1 mRNA: Expression of HO-1 mRNA in uninjured spinal cord was examined by RT-PCR. Relative levels of HO-1 mRNA were measured with real-time PCR before injury, and at 1, 3, 7, and 14 days after injury.

Distribution of HO-1 Protein in the Spinal Cord: Spinal cords were longitudinally cryosectioned at 10 µm in thickness. Sections from both mice were stained with anti-HO-1 antibody and neurofilament before injury and 3 days after injury. Statistical Analysis: Student’s t-test was used to assess the significance of differences between the two groups at each time point of assessment, and growth curve analysis was used to assess the chronological changes in each group.

Results
Functional Assessment and Electrophysiological Recording: BBB scores of the open field trials gradually improved from day1 to day14 after spinal cord injury in all mice. The mean BBB scores of the Bach1−/− mice were higher than those of the wild type mice throughout the observation period, although none of the differences were significant (Fig.2A). In the preinjury state, there was no significant difference between the average MEP amplitudes in both mice. After spinal cord injury, MEP amplitudes recovered from day1 to day14 in all mice. MEP amplitudes of the triceps, which were the transaction control, showed little change and no significantly differences were observed between the mice during the observation period. The peak-to-peak amplitudes were significantly larger, compared with respective controls, in Bach1−/− mice than in wild type mice throughout the observation period (Fig.2B).

Expression of HO-1 mRNA: HO-1 mRNA was increased significantly in both mice until 3 days after injury, and expressed at significantly higher levels in spinal cord in Bach1−/− mice as compared with the spinal cord from wild type mice at the every time of assessment (Fig.2C).

Discussion
In the electrophysiological study, the peak-to-peak amplitude of MEPs was significantly larger in Bach1−/− mice than in wild type mice from 1 day after injury. In Bach1−/− mice, HO-1 mRNA is expressed constitutively at high levels under normal physiological conditions, and expressed at significantly higher levels in spinal cord in Bach1−/− mice as compared with the spinal cord from wild type mice at the every time of assessment. So, HO-1 inhibits oxidative stress just after spinal cord 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 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.

Conclusions
These results suggest that HO-1 modulates the secondary injury process and high HO-1 expression may preserve spinal cord function in the early stages after spinal cord injury in Bach1−/− mice. Treatment that induces HO-1 expression at these early stages may preserve the functional outcome after spinal cord injury.

References