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Environmental enrichment reduces sclerostin and increases bone formation in brain-injured mice

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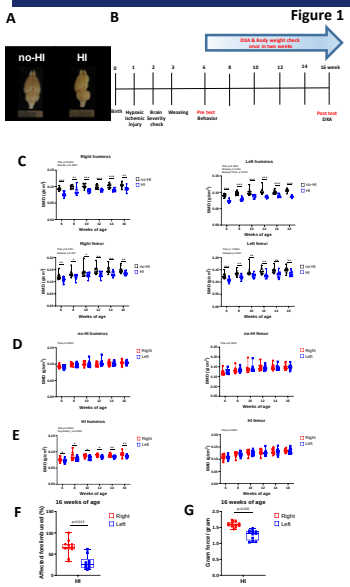
Abstract

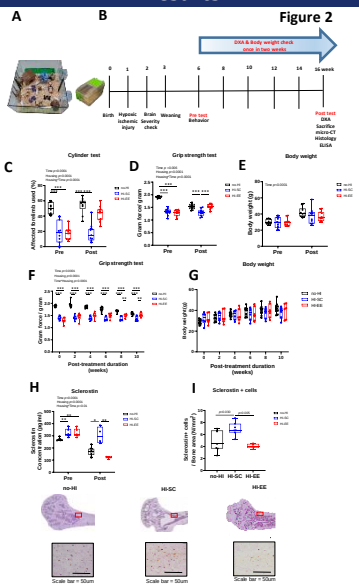
Cerebral palsy (CP) is a permanent neurological disorder influencing abnormal motor control as a complication of immature brain damage. Long-term limitations of mechanical loading can elevate sclerostin levels, leading to bone loss. Patients with CP are vulnerable to osteoporosis and risk of fractures. However, there is no optimal treatment for preventing bone loss with down-regulation of sclerostin in CP. Therefore, we aimed to determine the effects of environmental enrichment (EE) which provides sufficient weight-bearing as a rehabilitative exercise in chronic hypoxic-ischemic (HI) brain-injured mice with bone loss. Total 56 mice were included in this study. HI brain damage was induced in seven-day-old pups. No-HI mice (n=12) and HI mice (n=12) were used for the first experiment to verify differences of bone mineral density (BMD) and behavior between no-HI group and the HI group (Figure 1). Thereafter, to see whether EE positively affects bone formation, no-HI (n=10), HI-SC (n=11), and HI-EE (n=11) mice were used for the second experiment. At 6 weeks of age, mice were randomly assigned to no-HI group, HI-standard condition (SC), or HI-EE group for 10 weeks (Figure 2A,B). Mice in the EE group were housed in a large cage (86×76×31 cm^3) containing novel objects, running wheels for voluntary exercise, and social interaction was allowed. Forelimb use and muscle strength on the affected side were assessed by the cylinder and grip strength tests. Effects of EE on the regulation of sclerostin levels in serum and bone tissue were measured by enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry. Bone density was analyzed using dual-energy X-ray absorptiometry and micro-CT. Bone formation marker, procollagen type 1 N-terminal propeptide (P1NP) and bone resorption marker, C-terminal telopeptide of type 1 collagen (CTX-1) were detected using ELISA. There were differences in femur and humerus BMD as an indication of disease effects, showing significantly higher BMD in no-HI group than in HI group (Figure 1C). The behavioral analyses including affected forelimb use and grip strength revealed side-to-side differences, showing a functional loss in the affected limbs of HI mice (Figure 1F,G). This study revealed that the HI brain-injured mice exposed to EE showed significantly higher use of affected forelimb and muscle strength than those of HI mice living in SC (Figure 2C-G). The EE-induced functional improvements were associated with anti-sclerostin effects detected in serum and bone tissue (Figure 2H-J). A bone-forming effect was found with an increase of osteoblast number and P1NP in contrast to CTX-1 (Figure 3B,E). EE allowed brain-injured mice to experience sufficient mechanical loading, which helped improvement in bone mineral density through anti-sclerostin effects (Figure 3A,C,D). This study suggests that a rehabilitative environment providing sufficient weight-bearing would be helpful to reverse sclerostin levels for the treatment of bone loss in CP.

Materials & Methods

Pregnant CD-1 (ICR) mice (Orient Bio Incorporation, Seongnam, Korea) were used in this study. A surgical procedure for hypoxic-ischemic (HI) brain injury was performed in seven-day-old pups. In a supine position, the skin of the neck was carefully incised. The right common carotid artery (CCA) was then permanently ligated and the incision region was sutured under sufficient respiratory anesthesia with isoflurane. Finally, these pups were exposed to hypoxic environments (92% N_2, 8% O_2) for 90 minutes in a hypoxic chamber at 36–37.5 °C. No-HI mice had no surgical procedures including common carotid artery ligation or anesthesia. One week after surgery, the severity of the brain injury was visually checked through the semi-transparent skull after anesthesia. We described the criteria of HI brain injury. The brain damage with > 50% of the hemisphere was defined as severe injury (Figure 1A). To obtain HI brain injured mice with insufficient physical activities, mice with severe brain damage were used in this study.

Results





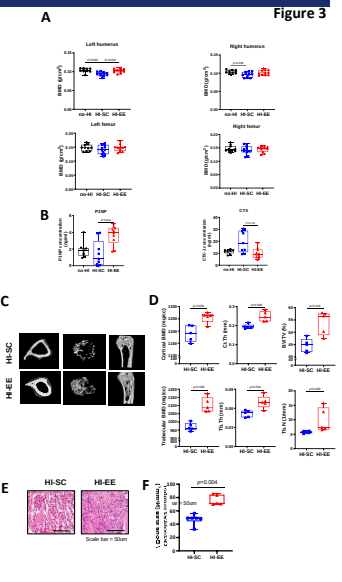


Figure 1. Bone loss and functional impairments from HI brain injury in mice (A) The degree of brain damage. (B) The timeline of the first experiment. (C) Bone mineral density of four limbs including humerus and femur between no-HI and HI brain-injured mice. HI brain-injured mice showed a significantly reduced bone density in four limbs when compared with no-HI by two-way repeated measures ANOVA, showing significant time effect and disease effect. The disease effect on each time point was clarified by Mann-Whitney U test. (D) No-HI brain injured mice showed a symmetrical bone density between both sides of humerus and femur. (E) However, HI brain injured mice had an asymmetrical bone density between affected side and less affected side of humerus by Wilcoxon signed-rank test. (F, G) Functional impairments of HI brain injured mice were identified with reduced forelimb use and grip strength in the affected side compared with less affected side by Wilcoxon signed-rank test.

Figure 2. Effects of environmental enrichment on behavioral functions and anti scleorstin effects in HI brain-injured mice (A) Housing conditions. (B) Second experiment timeline (C) Affected forelimb use was enhanced in HI-EE group compared to HI-SC group based on maintenance of time, housing interaction effect by two-way repeated measures ANOVA. Housing effect also evaluated by one-way ANOVA with Bonferroni post-hoc analysis. (D) Grip strength assessment showed similar statistical pattern. (E, G) Body weight excluding the effect of body mass between groups by supporting grip strength outcomes in this study. (F) In the grip strength, interaction effect found showing overall time and group effect by two-way repeated measures ANOVA. EE significantly increased the grip strength at 8 weeks and 10 weeks post treatment by one-way ANOVA with Bonferroni post-hoc analysis. (H) Anti-sclerostin effect after exposure to EE based on the time effect, housing effect, and interaction effect by two-way repeated measures ANOVA. Ten weeks after treatment, exposure to EE showed a significant decrease in sclerostin level by one-way ANOVA with post-hoc Bonferroni analysis. (I) Sclerostin protein measured in cortical bone from humerus by IHC. (J) Sclerostin level was downregulation in the affected humerus of HI-EE than HI-SC by one-way ANOVA with post-hoc Bonferroni analysis.

Figure 3. Bone formation with improved bone density by environmental enrichment. Comparison of BMD among no-HI, HI-SC and HI-EE was analyzed by one-way ANOVA. Based on a fundamental disease condition difference between no-HI and HI-SC, HI-EE significantly increased humerus BMD compared to controls, indicating housing effect by one-way ANOVA with Bonferroni post-hoc analysis. (B) Changes in bone turnover markers including bone formation marker (P1NP) and bone resorption marker (CTX-I) among no-HI, HI-SC and HI-EE supported bone forming effect by EE. (C) Representative micro-CT image of the humeral heads including cortical bone and trabecular bone in two groups; HI-SC and HI-EE at 16 weeks of age. (D) Micro-CT Parameters. Comparison of between HI-SC and HI-EE by Mann-Whitney U test. (E) Representative images of Hematoxylin/Eosin (H&E) staining of proximal humeral tissues between HI-SC and HI-EE. Osteoblast numbers per bone surface were assessed. (F) Osteoblast numbers per bone surface were assessed by Mann-Whitney U test. (***p<0.001; **p<0.01; *p<0.05)

Conclusion

From our significant bone-forming outcomes of EE, suppression of sclerostin, a therapeutic target, could be possible in HI brain injured mice with bone loss. A neuro-motor recovery based on voluntarily enhanced mechanical loading from EE induced anabolic effects overcoming severely aggravated bone health conditions in this CP model. As a preclinical step, our study calls for a comparative study of EE and anti-sclerostin treatment for fundamental clinical decisions in patients with CP.

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