Evaluation Of Behavior And Markers Of Pain In Osteoporotic Mice

Yohei Naito, Hiroki Wakabayashi, Takahiro Iino, Akihiro Sudo.
Mie University Graduate School of Medicine, Tsu, Japan.

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Introduction:
Osteoporotic patients with no evidence of fractures sometimes experience vague low back pain. A previous report has indicated that sensory innervation of osteoporotic rat vertebrae showed increased expression of Transient receptor potential vanilloid 1 (TRPV1) and calcitonin gene-related peptide (CGRP) in dorsal root ganglion (DRG) neurons\(^1\). However, there have been few reports regarding the correlation between osteoporosis and pain-related behavior. The objective of the current study was to investigate pain-related behavior and pain markers in osteoporotic mice.

Methods:
Female ddY mice (8 weeks old) were either ovariectomized bilaterally under anesthesia with pentobarbital sodium intraperitoneally (OVX group) or sham-operated (ovaries exteriorized but not removed; SHAM group). The proximal tibial metaphyses were analyzed three-dimensionally by micro-computed tomography (μCT) 4 weeks after surgery (OVX group; 8 hindlimbs, SHAM group; 8 hindlimbs). Mechanical sensitivity was tested using von Frey filaments 4 weeks after surgery. The frequency of the withdrawal response and the withdrawal threshold to the application of von Frey filaments to the planter surface of the hindpaws was examined. To evaluate the frequency of the withdrawal response, two von Frey filaments with forces of 0.4 and 1.0 g were applied 10 times each in ascending order of force, and the number and intensity of withdrawal responses were noted. Results were expressed as the percent response frequency of paw withdrawals. To evaluate the withdrawal threshold, each von Frey filament was applied once, starting with 0.008g and increasing until a withdrawal response was reached, which was considered a positive response. The lowest force producing a response was considered the withdrawal threshold. TRPV1 and CGRP expression in L5 DRG neurons were examined 4 weeks after surgery using immunohistochemistry (OVX group; 8 DRGs, SHAM group; 8 DRGs). The ratio of TRPV1-, CGRP-immunoreactive cells to total DRG neurons was counted and averaged for each DRG. Statistical analysis was performed using Mann-Whitney U-test and p < 0.05 was accepted as indicating significance.

Results:
μCT analysis of the proximal tibial metaphysis revealed bone loss in the OVX mouse compared with the SHAM mouse (Fig. 1). Bone volume/tissue volume (BV/TV) (Fig.2) and trabecular number (Tb.N) were significantly less in the OVX group than in the SHAM group, whereas trabecular separation (Tb.Sp) was significantly greater in the OVX group than in the SHAM group. The paw-withdrawal-frequency stimulated by von Frey filaments with strength of 0.4 and 1.0 g was significantly higher in the OVX group than in the SHAM group (Fig.3). The withdrawal threshold was significantly lower in the OVX group than in the SHAM group (data not shown). Immunohistochemical analysis showed that the ratio of TRPV1-, CGRP-immunoreactive DRG neurons in the OVX group was significantly higher than in the SHAM group (Fig 4, 5).

Discussion:
The ovariectomized animal model is widely used in the study of post-menopausal osteoporosis\(^2\). In this study, ovariectomy induced significant bone loss in the hindlimbs of mice. The application of von Frey filaments to the planter surface of the hindpaw is often used to evaluate hindlimb hyperalgesia (e.g., knee joint inflammation\(^3\), tibia fracture\(^4\), etc.). In this study, mechanical hyperalgesia was observed in osteoporotic mice. TRPV1 is a ligand-gated nonselective cation channel, which can be activated by capsaicin and other stimulation such as noxious heat\(^5\) and low pH\(^6\). L Yu et al reported that TRPV1 in distinct subtypes of DRG neurons plays a role not only in the acute, but also in the chronic inflammatory pain, and that mechanical allodynia exists in chronic inflammatory pain, in which TRPV1 may also take effect\(^7\). CGRP has been reported to produce hyperalgesia via both Protein kinase A and C second-messenger pathways, thus elevated CGRP expression is suggested to produce pain\(^8\). In this study, upregulation of TRPV1 and CGRP expression was recognized in DRG neurons innervating osteoporotic mouse hindlimbs. The upregulation of pain markers might induce mechanical hyperalgesia in osteoporotic mouse hindlimbs.

Significance:
The ovariectomy mice induced bone loss and mechanical hyperalgesia in hindlimbs with upregulation of TRPV1 and CGRP expression in DRG. The results suggest that osteoporotic pain may correlate with TRPV1 and/or CGRP expression in sensory
innervation.

Acknowledgments:

References:
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