Introduction: Several reports indicated that interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) play important regulatory roles in bone remodeling and homeostasis. In addition, receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) have been shown to be important regulators of osteoclastogenesis during bone remodeling, and their expressions were examined during fracture healing in a mouse model of tibial fracture. However, studies linking RANKL, OPG, IL-6 and TNF-α in patients with head injury and fracture are lacking.

Materials and Methods: Subjects

24 male patients of head injury and fracture with a mean age of 42.0 (24-59) and 26 male patients of fracture alone with a mean age of 42.4 (29-58) were enrolled at the authors’ institution. Patients who had diabetes, steroids or bisphosphonate medication and a previous head injury or bone related pathology were excluded from the study. 40 healthy men with a mean age of 40.7 (27-58) were recruited to serve as controls.

The Glasgow coma scale (GCS) was calculated for each patient with a head injury on arrival, within the first few hours of admission to hospital, and at 4, 8 and 12 weeks after injury. 20 ml of peripheral blood were obtained from the 24 patients with head injury and fracture and from the 26 patients with fracture only. In all patients with fracture, surgery was performed. The mean period to operation in patients with head injury and fracture and in patients with fracture only were 8.2 days (3-14) and 3.4 days (1-12), respectively.

Measurements of the serum levels of RANKL, OPG, IL-6 and TNF-α.

RANKL levels were measured in serum by a sandwich ELISA (Immundiagnostik, Bensheim, Germany). The detection limit was 1.56 pg/ml. The intra-assay CV for RANKL measurement was 3-5% and interassay CV was 6-9%.

Serum OPG levels were measured by sandwich ELISA (Immundiagnostik, Bensheim, Germany) method. The assay includes two highly specific antibodies against OPG. The antibody is capable of neutralizing the biological activity of recombinant human OPG. The detection antibody was a biotin-labelled polyclonal anti-human OPG antibody derived from a goat, immunized with human recombinant OPG. The concentrations of serum OPG were calculated on the basis of the protein concentration as described by the manufacturer. The detection limit was 2.8 pg/ml. The intra-assay coefficient of variation (CV) for OPG measurement was 8-10% and interassay CV was below 10%.

IL-6 and TNF-α were measured by quantitative ELISA (Quantikine, R&D systems, Minneapolis, MN). The minimal detectable amounts of IL-6 and TNF-α were 8 pg/ml and 15.6 pg/ml, respectively.

Results: Age was found to have no correlation with serum RANKL, OPG, IL-6 or TNF-α levels and no significant difference was found between the three groups (head injury and fracture, fracture only and normal controls, referred to below as the head injury, fracture, and control groups, respectively) with respect to age.

Serum levels of RANKL

RANKL levels were significantly higher in the head injury group immediately after injury than in controls (p < 0.05), however, RANKL levels were significantly lower in the head injury group at 4, 8 and 12 weeks after injury than in controls (p < 0.05, p < 0.05 and p < 0.05, respectively). Moreover, RANKL levels were significantly higher in the fracture group immediately after injury than in controls (p < 0.05), but no significant differences were observed between these two groups at 4, 8 and 12 weeks after injury. No significant differences were observed between RANKL levels in the head injury and fracture groups immediately after injury or at 4 weeks after injury. However, RANKL levels were significantly lower in the head injury group than in the fracture group at 8 and 12 weeks after injury (p < 0.05 and p < 0.05, respectively).

Serum levels of OPG

OPG levels were significantly higher in the head injury group than in the controls immediately after injury and at 4 and 8 weeks after injury (p < 0.05, p < 0.05 and p < 0.05, respectively), but no significant difference was observed between levels in these two groups at 12 weeks after injury. Moreover, OPG levels were significantly higher in the fracture group than in the controls immediately after, and 4 weeks after injury (p < 0.05 and p < 0.05, respectively), but no significant difference was observed between these two groups at 8 and 12 weeks after injury. No significant differences was observed between the head injury and fracture groups immediately after injury, but OPG levels were significantly higher in the head injury group than in the fracture group at 4, 8 and 12 weeks after injury (p < 0.05, p < 0.05 and p < 0.05, respectively).

RANKL/OPG ratios

RANKL/OPG ratios were significantly lower in the head injury group than in the controls immediately after and at 4, 8 and 12 weeks after injury (p < 0.05, p < 0.05, p < 0.05 and p < 0.05, respectively). RANKL/OPG ratios were significantly lower in the fracture group than in the controls immediately after and 4 weeks after injury (p < 0.05 and p < 0.05, respectively), but no significant difference was observed between these two groups at 8 and 12 weeks after injury. RANKL/OPG ratio were significantly lower in the head injury group than in the fracture group at 8 and 12 weeks after injury (p < 0.05 and p < 0.05, respectively), but no significant differences was observed between these two groups immediately after and at 4 weeks after injury.

Serum levels of IL-6

IL-6 levels were significantly higher in the head injury group than in the controls immediately after and at 4, 8 and 12 weeks after injury (p < 0.05, p < 0.05, p < 0.05 and p < 0.05, respectively), and were significantly higher in the fracture group than in the controls only immediately after injury (p < 0.05). No significant difference was found between the head injury and fracture groups immediately after injury, but IL-6 levels were significantly higher in the head injury group at 4, 8 and 12 weeks after injury (p < 0.05, p < 0.05 and p < 0.05, respectively).

Discussion: We have shown changes in the profiles of RANKL, OPG and IL-6 levels and RANKL/OPG ratios and propose a mechanism by which altered repair of a fracture may occur in patients with head injury. Moreover, these findings will indicate that RANKL, OPG and IL-6 levels, and RANKL/OPG ratios could stimulate the stages of repair of a fracture. However, further in vitro and in vivo studies are required to confirm the validity of the proposed mechanism.


Acknowledgements: This study was supported by Medical Research Institute Grant (2007-12), Pusan National University.