PATHOMECHANISM OF DELAY IN FRACTURE HEALING BY CYCLOOXYGENASE-2 INHIBITOR WITH REFERENCE TO VASCULAR ENDOTHELIAL GROWTH FACTOR

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INTRODUCTION
Recently, cyclooxygenase-2 (cox-2) specific inhibitors have widely used to alleviate pain for patients with bone fractures. However, the effects of specific cox-2 inhibitor on the fracture healing have been unclear yet. Fracture healing is initiated by an inflammatory response expressing variety of cytokines and growth factors. Vascular endothelial growth factor (VEGF) is one of such cytokines expressing at this phase in the vicinity of the fracture site, and angiogenesis is induced for fracture healing. The purpose of this study is to investigate the effects of etodolac, a cox-2 specific inhibitor, on fracture healing and to discuss its pathomechanism with reference to expression of VEGF at the fracture site.

MATERIALS AND METHODS
Animal fracture model:
Female 18–week-old Wister rats, weighing 250–300 (g), were used in this study. This study conformed to the guidelines for the care and use of laboratory animals of our university. The rats were randomly assigned to two groups; the first group rats received etodolac (a cox-2 specific inhibitor; Nippon Shinyaku Co. Ltd, Tokyo, Japan) as a dose of 20 mg/kg body weight every day (E group), and the second group rats were in the vehicle control group (V group). The drug was administered intraperitoneally. According to the three point bending methods proposed by Bonnarens and Einhorn (2), closed non-displaced mid shaft femoral fractures were created under general anesthesia.

(STUDY I)
Radiographic and biomechanical evaluation:
Immediately after surgery, one-, two- and three- weeks following the surgery, anteroposterior radiographs were taken. With those radiographs, callus formation and bone union was evaluated. Radiographic scoring system for fracture healing was used for the evaluation (1). Three point bending test was used for the biomechanical evaluation using the MTS system (Test star-II, MTS Inc. Minneapolis, MN, USA). The load displacement curve was obtained from each specimen; the ultimate strength at failure and stiffness until failure were then calculated from the curve.

(STUDY II)
Reverse transcription-polymerase chain reaction (RT-PCR) for VEGF
Rats were killed 4 and 7 days after the fracture, and the fracture callus was obtained. To evaluate the expression of VEGF at the mRNA level, RT-PCR was used. The values were normalized using the value of beta-actin for semi-quantitative analysis.

RESULTS
(STUDY I) Figure 1 demonstrates weekly changes of the radiographical score in each group, and the score in E group was significantly lower than that in V group (p<0.05). The results indicated that bone union and callus formation were delayed by administration of etodolac. The data obtained from the biomechanical testing is shown in Figure 2. The ultimate strength was 41.2 and 95.1(N) for E and V group, respectively. The stiffness in E group was 30.4, and was 118.6 (N/mm) in V group. All mechanical parameters in the V group were significantly higher than those in the E group (p<0.05).

(STUDY II) In the fracture callus, mRNA of VEGF was revealed to express in both groups. The expression in the cox-2 treated rats was down-regulated at approximately half of that in the vehicle control rats 4 and 7 days after the fracture.

DISCUSSION and CONCLUSION
In the present study, the effects of cox-2 specific inhibitor on fracture healing were evaluated. Weekly radiographs demonstrated that the callus formation surrounding the fracture site and bone union was poor in the cox-2 treated rat group. Furthermore, mechanical maturation, evaluated by bone strength and stiffness was delayed. Thereby, it was revealed that the cox-2 specific inhibitors inhibited the maturation of callus and delayed the fracture healing.

At the beginning of the fracture healing, an inflammatory response is initiated. During the inflammatory period, variety of cytokines and growth factors such as IL-1, -6, TNF-alpha, TGF-beta and FGFs are reported to express for the subsequent bone healing. One of the possibilities of the delayed healing is proposed to be that; the cox-2 specific inhibitors may inhibit those growth factors which are required for fracture healing. VEGF, the cytokine, which is regulating angiogenesis, was reported to express at the early phase of the fracture healing (3). In this study, the mRNA of VEGF was expressed in the callus at the fracture site in both cox-2 treated and vehicle control rat groups. It was shown to down-regulate with the administration of cox-2 inhibitor.

In conclusion, this preliminary study indicated that cox-2 specific inhibitor inhibits the fracture healing, and the delay in healing is probably caused by suppression of angiogenesis of the fracture callus by the drug.

REFERENCES