

## Cyclooxygenase-2 inhibitor delays fracture healing in rats

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**Background** Cyclooxygenase-2 (COX-2) inhibitors have been reported to delay fracture healing. To investigate the major inhibitory period of COX -2 inhibitors in fracture healing, we administrated etodolac, a COX-2-specific inhibitor, to a rat fracture model by altering the period of administration from early to late.

**Method** After closed fractures had been created at the middle of the femoral shafts in 12-week-old Wister rats, a standardized dose of etodolac was administrated in three ways: group I received it for 3 weeks, group II for just the first week after operation, and group III for just the third (final) week. Group IV was the vehicle control group. Bone maturation was estimated by radiographic scoring system, and mechanically by a three-point bending test.

**Results and interpretation** In both the radiographic and mechanical studies, groups I and II showed lower scores than group IV, indicating that even a short period of administration of a COX-2-specific inhibitor in the early phase of fracture healing creates a risk of delayed healing.

The process of fracture healing can be divided into three phases: inflammatory, reparative, and remodeling. In the inflammatory phase, many cytokines, including cyclooxygenase (COX), specific growth factors, have been reported to arise at the fracture site (Einhorn et al. 1995, Gerstenfeld et al. 2001) and proceed to intramembraneous ossification. COX-1 is constitutively expressed in a variety of cells and tissues. Another isoform, COX-2, is the product of an immediate early response gene in

inflammatory cells and its induction has been proposed to be essential in early-stage fracture healing (Einhorn et al. 1995, Zhang et al. 2002, Gerstenfeld et al. 2003).

For the management of acute fractures, non-steroidal antiinflammatory drugs (NSAIDs) have been widely used as painkillers. They have been reported to have an inhibitory effect on cyclooxygenases (Altman et al. 1995). Recently, COX-2 inhibitors have been widely used because of their low risk to the gastrointestinal tract. However, it has also been reported that COX-2 inhibitors delay fracture healing (Endo et al. 2002, Simon et al. 2002, Gerstenfeld et al. 2003). Inflammation at the fracture site is essential for fracture healing and is usually self-limiting in nature; thus, even a short period of administration of a COX-2 inhibitor may have an attendant risk of delay in healing.

In our study we used etodolac, a selective COX-2 inhibitor, as a COX-2-specific NSAID. The COX-2 selectivity of etodolac is reported to be similar to that of celecoxib (Warner et al. 1999). In this study, we aimed to elucidate how the early administration of etodolac to a closed femoral shaft fracture of a rat causes disturbances in fracture healing.

### Animals and methods

#### *Animals and fracture model*

Twenty 12-week-old Wistar rats weighing 250–300 g were obtained from Charles River Japan Co. Ltd. (Yokohama, Japan). The rats were housed individually in a temperature-controlled ( $23 \pm 2^\circ\text{C}$ ) and

humidity-controlled ( $55 \pm 10\%$ ) room with a 12-hour light-dark cycle, and had free access to food and water. The study conformed to the guidelines for the care and use of laboratory animals at our university.

After rats were anesthetized with sodium pentobarbital (50 mg/kg body weight), skin, subcutaneous tissue, and the capsule of the knee joint were incised and the intertrochanteric space of the distal femur was exposed by a medial patellar approach. A 0.8-mm K-wire was inserted between the condyles to the bilateral femurs from the distal end. The capsule of the knee joint, subcutaneous tissue, and skin were sutured layer to layer with 4-0 silk.

Closed mid-shaft femoral fractures were created at both sides according to the three-point bending method proposed by Bonnarens and Einhorn (1984).

#### *Intraperitoneal administration of COX-2 inhibitor*

Rats were randomly assigned to one of four groups involving differences in the drug administration protocol: 5 rats received intraperitoneally administered etodolac (Nippon Shinyaku Co. Ltd, Tokyo, Japan) at a dose of 20 mg/kg body weight per day for 3 weeks (group I); 5 other rats had the same dose from operation to the 7th day—the early administration group (group II); and 5 others had the same dose from days 14 to 21—the late administration group (group III). The 5 remaining rats constituted the vehicle control group (group IV), and received injections of a 0.5% methyl cellulose solution.

#### *Radiographic evaluation*

Immediately after surgery, and 1, 2 and 3 weeks after surgery, postero-anterior radiographs of the femurs were taken to evaluate callus formation and bone healing, and they were evaluated on a scoring system for fracture healing (An et al. 1999). The radiographic scoring system consists of three categories: periosteal reaction, bone union, and remodeling. The full score for each category is 3 for category periosteal reaction, 3 for category bone union, and 2 for remodeling. For evaluation, the radiographs were scored by two orthopedic radiologists with randomized blinded selection of films. 3 weeks after surgery, the rats were killed with sodium pentobarbital, the femurs were

harvested from all rats, and all soft tissue and K-wires were removed. Unilateral femurs in each rat were randomly selected and stored at  $-20^{\circ}\text{C}$  until mechanical testing.

#### *Mechanical measurements*

Specimens were thawed to room temperature one day before mechanical testing, and were kept moist throughout the experiment. Evaluations were made using a three-point bending test as reported by Nakamura et al. (1989). Specimens were placed on a special metal holding device with supports located at a distance of 13 mm, and the device was connected to an actuator of the MTS system (Test Star-II, MTS Inc., Minneapolis, MN). The bending force was applied midway between the supports on the anterior surface at a speed of 10 mm/min until failure. Mechanical parameters were determined from the load-deformation curve, and the maximum load (N) at failure was determined as the ultimate force. The slope of the load deformation curve between 20% and 70% of the maximum load value was determined in the ascending portion as the stiffness of the femur (N/mm).

#### *Statistics*

For analysis of the radiographic scoring system and for evaluation of data from the mechanical testing, we applied both one-way ANOVA and Fisher's protected least significant difference test, which is one of the post-hoc tests for evaluation of the radiographic score. P-values less than 0.05 were considered to be significant.

## **Results**

#### *Radiographic evaluation*

20 right femurs from 20 rats were available for the radiographic evaluation using the scoring system. At 3 weeks, we observed bone union in groups III and IV, while bone union was poor in groups I and II (Table 1). Statistical evaluation was performed using the radiographs taken 3 weeks after operation. A one-way ANOVA analysis showed significant difference ( $p < 0.05$ ). A marked decrease in the scores was seen in groups I and II. The radiographic score of group I was lower than those of groups III and IV, and that of group II was also

Table 1. Data on radiographic scores of the various groups

Drug administration	Radiographic score			n
	mean	SD		
Group I: Weeks 1–3	2.4	0.6		5
Group II: Early, week 1	2.6	0.6		5
Group III: Late, week 1	4.4	2.2		5
Group IV: Vehicle control	5.6	1.3		5

Table 2. Comparison of the data for radiographic score between various groups: p-values from the Fisher's protected least significant difference test

	Group I	Group II	Group III	Group IV
Group I	–	0.8	0.03	0.002
Group II	–	–	0.05	0.003
Group III	–	–	–	0.2
Group IV	–	–	–	–

Table 3. Data on maximal strength from three-point bending test

Drug administration	Maximal strength (N)			n
	mean	SD		
Group I: Weeks 1–3	42.7	30.7		5
Group II: Early, 1 week	42.8	15.0		5
Group III: Late, 1 week	78.6	13.3		5
Group IV: Vehicle control	94.8	31.7		5

Table 4. Comparison of the data for maximal strength between various groups: p-values from the Fisher's protected least significant difference test

	Group I	Group II	Group III	Group IV
Group I	–	0.1	0.0003	<0.0001
Group II	–	–	0.009	0.0008
Group III	–	–	–	0.3
Group IV	–	–	–	–

lower than those of groups III and IV (Table 2). The results indicated that bone union and callus formation had both been delayed by the administration of etodolac for just 1 week in the rat fracture model animals.

Table 5. Data on stiffness from the three-point bending test

Drug administration	Stiffness (N/mm)			n
	mean	SD		
Group I: Weeks 1–3	53.6	71.0		5
Group II: Early, 1 week	31.5	18.6		5
Group III: Late, 1 week	101.7	25.0		5
Group IV: Vehicle control	118.2	49.8		5

Table 6. Comparison of data for stiffness between various groups: p-values from the Fisher's protected least significant difference test

	Group I	Group II	Group III	Group IV
Group I	–	0.1	0.004	0.002
Group II	–	–	0.1	0.05
Group III	–	–	–	0.7
Group IV	–	–	–	–

### Mechanical evaluation

For the mechanical evaluations, unilateral femurs were selected randomly. Ultimate strengths were measured by the three-point bending system (Table 3). The ultimate strength for group I was less than for groups III and IV; and that for group II was less than for groups III and IV (Table 4).

In addition, the stiffness values were determined by the three-point bending system (Table 5) and showed the same tendency as in the ultimate strength values. Stiffness for group I was lower than for groups III and IV, and that for group II was lower than for group IV (Table 6).

### Discussion

In the treatment of bone fractures, analgesic agents such as NSAIDs have been widely used for pain control and have been reported to delay fracture healing (Elves et al. 1982, Altman et al. 1995, Brown et al. 2004). It has been proposed recently that COX-2 inhibitors delay fracture healing to a greater extent than COX-1 inhibitors.

At the start of fracture healing, an inflammatory response is initiated; however, during the inflammatory period, a variety of cytokines and growth fac-

tors such as IL-1, IL-6, TNF- $\alpha$ , TGF- $\beta$  and FGFs are reported to be expressed, to assist in the subsequent bone healing (Jingushi et al. 1990, Einhorn et al. 1995, Gerstenfeld et al. 2001). One possibility that has been proposed for the delayed healing is that COX-2-specific inhibitors may inhibit the expression of those growth factors that are required for fracture healing. BMP-2 is a member of the TGF- $\beta$  superfamily, and is a crucial growth factor for bone formation. Koide et al. (1999) demonstrated that BMP-2 could stimulate osteoclast-like multinucleated cell formation in the presence of IL-1 $\alpha$ , and that adding the COX-2-specific inhibitor NS-398 abolished the stimulation. Zhang et al. (2002) examined the effects of COX-1 and COX-2 on bone repair using COX-1 and COX-2 knockout mice, and found delayed endochondral bone formation in the COX-2 knockout mice compared with that in the COX-1 knockout mice. In another study, 3-week administration of etodolac delayed fracture healing in a rat model (Endo et al. 2002). However, when etodolac had caused most of the delay in fracture healing was not elucidated.

Our radiographic and mechanical studies have revealed similar results for fracture healing in rats—for those having etodolac administration throughout the experimental period and for those having etodolac only in the early phase. Moreover, since COX-2 was scarce 2 weeks after fracture, rats receiving COX-2-specific inhibitor administration for just the late phase of the period showed similar results to those in the vehicle control group. Thus, we have shown that there is a time dependency in the effect of COX-2-specific inhibitors on fracture healing.

One of the weaknesses of our study was the small number of specimens. However, we feel that the use of randomization in such a small population helps to achieve a reasonably balanced representation.

In conclusion, we have shown that a COX-2-specific inhibitor delayed fracture healing, and its effects were more extensive in the early phase. Our findings provide evidence that this kind of analgesic should be used with care during the initial phase of the healing process following bone fracture.

No competing interests declared.

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