Effects of a Cyclooxygenase 2 Inhibitor on Fracture Healing in a Rat Model

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Abstract

Since the advent of cyclooxygenase 2 (COX-2) inhibitors, little research has been done on the effects of these medications on fracture healing.

In the study reported here, we sought to determine whether a COX-2 inhibitor, celecoxib, affects strength and amount of healing callus formed after a fracture. Forty-eight male Sprague-Dawley rats were evaluated for impairment of fracture healing with celecoxib use. Compared with controls, celecoxib-treated rats had a significant decrease in force required for refracture (P = .0199). We do not recommend routine use of celecoxib in postfracture pain control, particularly when fracture union is tenuous.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been one of the mainstays of treatment for pain after surgery and in patients with fractures or soft-tissue injuries. The influence of NSAIDs on bone metabolism has been well documented. There is evidence supporting and refuting the notion that NSAIDs inhibit bone healing.[1-5] However, some authors believe that differences in protocol methodology (eg, drug dosage, dosing rates, timing and method of evaluating fracture healing) account for some of the variations in study outcomes.[6]

Several investigators have reported on the inhibitory effects of NSAIDs on bone formation, bone remodeling, and mineralization in animal models.[7-15] NSAIDs have been shown to induce qualitative histologic changes in the healing process. Immaturity of histologic grade of callus formation with NSAID use was noted early during the inflammatory process and continued throughout the course of healing.[3,6,16] Local blood flow to the site of an osteotomy was shown to be markedly impaired early in the course of healing.[17] NSAIDs have also been shown to decrease mechanical rigidity and the bending moment at a fracture or osteotomy site.[6,14,18,19] Many investigators have reported no difference in quantitative amount of direct or radiographically measured callus formed during NSAID use.[2,19,20] However, as testing in these studies demonstrated significantly decreased mechanical strength of healing callus with NSAID use, it may not be advisable to rely solely on radiographs to demonstrate completion of healing.

With respect to fracture healing, cyclooxygenase 2 (COX-2) inhibitors have not been scrutinized as much as nonselective NSAIDs have. In the study reported here, we sought to examine the effects of a COX-2 inhibitor, celecoxib, on mechanical strength and amount of callus formed in a fracture model. As COX-2 inhibitors (like nonselective NSAIDs) inhibit the inflammatory process, we hypothesized that we would find a decrease in the mechanical strength of callus and a quantitative difference in the amount of callus.

Materials and Methods

Fifty male Sprague-Dawley rats weighing approximately 350 g were acclimated to their environment with food and water for 7 days before surgery. The animals were maintained with standard laboratory feed and water for the duration of the experiment. All surgery, recovery, and housing of the rats in this study were conducted at the Wright State University Animal Laboratory Facility.

Each rat underwent a surgical procedure after anesthesia was applied. Ketamine 35 mg/kg plus xylazine 5 mg/kg intraperitoneal was used for induction. Inhaled isoflurane was used for maintenance anesthesia. Once the rat was anesthetized, its right knee and back (just posterior to the scapulae) were shaved and surgically scrubbed. A 21-gauge needle (0.9 mm in diameter, 2.5 cm in length) was inserted percutaneously into the intramedullary canal of the femur in a retrograde fashion. A fracture was then accomplished without violating the skin using a jig and a pneumatic device. The ends of the femur were supported laterally while the pneumatic device was applied to the medial aspect of the femur to cause a mid-diaphyseal fracture. An anteroposterior radiograph was then obtained of the affected limb under anesthesia to ensure that a transverse, mid-diaphyseal fracture was created. A small incision was made in the area prepared on the back of the neck, and a subcutaneous pocket was created with a hemostat. An Alzet 2001 osmotic pump (200 uL volume) filled with sufentanil (2.5 µg/mL) was placed subcutaneously in this space. The wound was then closed with staples.

Postoperative care consisted of routine wound checks and evaluation of behavior, including motion and appetite.

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Figure 1. Mean load to failure for control and treatment (celecoxib) groups of rats.

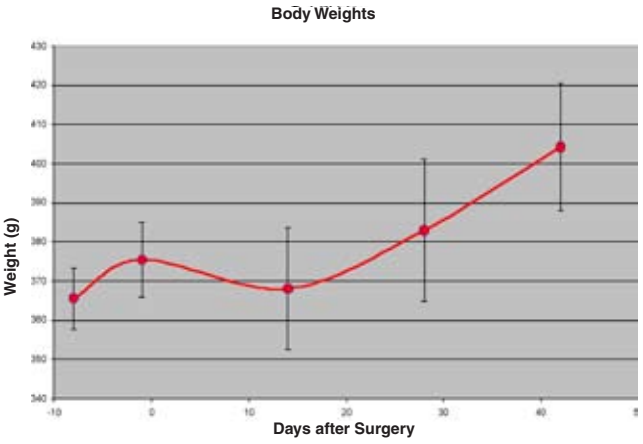

Figure 2. Mean weights of rats over course of study.

Animal weights were obtained on arrival at the laboratory, at time of surgery, and every 2 weeks thereafter. The rats were not immobilized after surgery. Pain management consisted of sufentanil administered by osmotic pump for 1 week at a rate of approximately 1 μL/h.

Beginning on the day of surgery, rats were placed in the control group or the treatment group. The control group received water and food without any COX-2 inhibitor.

The effective daily dose of celecoxib in rats is 5 to 30 mg/kg. The amount of rat chow consumed daily by a rat of the size used in this study is 32 g.[21] For the treatment group, the drug was administered by grinding a 100-mg tablet of celecoxib into 1 kg of feed and mixing thoroughly. Treatment group rats received celecoxib at a mean of 3.2 mg/d, well within the 1.6- to 10-mg/d dosing range. This delivery method and this dosing range have been used in similar studies.[21]

Rats were housed 2 per cage during the study, and limitations on their activity after the index procedure were minimal. Soon after the procedure, 2 of the 50 rats were euthanized (see Results). Osmotic pumps were removed between postoperative days 7 and 10, per manufacturer recommendation. Each of the remaining 48 rats received ketamine 35 mg/kg plus xylazine 5 mg/kg intraperitoneal for induction and maintenance with inhaled isoflurane. The site of the pump was prepared in a similar manner to the initial surgery, with shaving and surgical scrubbing. Staples were removed and the incision reopened. The osmotic pump was removed, and the wound was closed once again with staples. This procedure produced very minimal discomfort in the animals, and no additional pain medication was required.

Twelve animals (6 control, 6 treatment) were randomly selected for sacrifice (by carbon dioxide overdose) at each of 4 intervals (2, 4, 8, and 12 weeks). Fractured femurs were removed en bloc, and all soft tissue was carefully dissected free. Calipers were used to measure the length and maximum width of the callus. Specimens were set aside in moist physiologic saline solution and refrigerated until mechanical testing. Before mechanical testing, a needle driver was used to remove the intramedullary needle. A 3-point bending technique was used in measuring the load-bearing capability of the healing bone. Each specimen was supported on 2 parallel bars with the callus in the center; a constant load was applied until fracture occurred. The load/deformation data were acquired and the load at failure recorded.

The *t* test was used to compare the control and treatment groups with respect to amount of force needed for fracture and cross-sectional elliptical area. We planned for a 20% difference in mean amount of force needed for fracture (control, 100 N; treatment, 80 N) and considerable variability among animals in amount of force (ie, 75% of anticipated group means: control, 75 N; treatment, 60 N). With α at .05, β at .20, and power at 80%, 15 rats per group were needed. Thus, we had an adequate sample size for the overall comparison between the 2 groups (actual sample size per group, 24) but inadequate power for comparisons of the sacrifice intervals (2, 4, 8, and 12 weeks). The practicalities of our study and its limited budget permitted only 6 animals per interval. Inferences were made at $P = .05$. All analyses were conducted with SAS version 8.2.

Results

We began the study with 50 rats. Soon after the index procedure, 1 rat had an intramedullary needle misplaced in the soft tissues and then a fracture created. After multiple failed attempts to blindly insert the intramedullary needle past the fracture site, we had to euthanize this rat. Another rat had adequate intramedullary fixation, but a comminuted fracture pattern was found on postfracture radiographs. This rat also was euthanized. The remaining 48 rats were divided into 2 groups, control (n = 24) and treatment (n = 24).

Figure 1 shows mean load to failure for the control and treatment groups. Mean force needed to break femurs in the treatment group (70.476 N) was significantly ($P = .02$) lower than that in the control group (91.190 N) when taken across all healing times. Mechanical load comparisons between control and treatment groups at the 4 sacrifice intervals (2, 4, 8, and 12 weeks) were not significant.

In 6 of the 48 rats, the fracture remodeled enough that

fracture height could not be accurately determined with calipers. Two of these 6 rats were from the week 8 control group, 2 were from the week 12 control group, and 2 were from the week 12 treatment group. Subtracting these 6 rats left 42 (20 control, 22 treatment) for analysis of the cross-sectional elliptical area. Callus area (A) was calculated as an elliptical area in which $A = \Pi \times \text{height}/2 \times \text{width}/2$. In the overall comparison, there was no statistically significant difference in mean cross-sectional elliptical area between the control group (162.71 mm^2) and the treatment group (155.77 mm^2). In addition, none of the weekly interval comparisons was significant for cross-sectional area.

Weight data appear in Figure 2. The rats gained weight during the acclimation period and, as a whole, lost weight by the 2-week postoperative weighing. We believe the weight loss is secondary to increased metabolic need after surgery. On daily recorded observations, the rats were noted to be moving with minimal discomfort and eating regularly. Drug delivery may have been compromised during the 2 weeks after surgery, but that explanation is less plausible given the noticeable difference in fracture strength.

Discussion

NSAID use is extremely common in the treatment of patients with fracture, soft-tissue injury, and postoperative pain. Narcotic medications are used acutely for pain in these situations as well, but they are often supplemented with an NSAID. To guard against dependency and abuse, many physicians try to limit their patients' narcotic use. Often, pain persists after narcotics are stopped, and patients are continued or started on an anti-inflammatory medication for the many weeks of fracture healing. However, many studies have shown that NSAID use delays and impairs fracture healing.[6-8,10,13-20,22]

Use of anti-inflammatory medications during fracture care remains a clinical concern. Reports of nonunion and delayed union of fractures are abundant.[23] Study results that show NSAID-related decreases in spinal fusion[24,25] and femoral shaft union rates[26] have changed the practice habits of many orthopedic surgeons, such that they avoid NSAID use during fracture care.

Celecoxib was the first drug in a relatively new class of NSAIDs, the COX-2 inhibitors. Nonselective NSAIDs block both COX-2 and cyclooxygenase 1 (COX-1), whereas these new selective medications target only COX-2. COX-1 is found in almost all tissues and appears to provide homeostatic control for prostaglandin levels in tissues but does not have a role in inflammation.[21,27] COX-2 is an inducible enzyme found in tissues and is produced only at inflammation sites.[28,29] The obvious advantage of COX-2–specific inhibitors is their ability to block inflammation without disrupting the normal homeostatic functions of prostaglandins throughout the body. In several studies, celecoxib has been shown to provide arthritis patients with pain relief comparable to what nonselective NSAIDs provide, but without increased incidence of gastrointestinal or bleeding complication.[30-34] Celecoxib and other COX-2 inhibitors have been used for acute pain after surgery and in patients with fractures or soft-tissue injuries. There is some indication that COX-2 inhibitors may adversely affect ligament healing.[21]

Since the advent of COX-2–selective inhibitors, however, not much research has been conducted on the effect of these medications on fracture healing. Three COX-2 inhibitors have been introduced for clinical use: celecoxib, rofecoxib, and valdecoxib. Recently, rofecoxib was removed from the market because of reported cardiac side effects. Celecoxib preferentially inhibits the cyclooxygenase activity of COX-2 with approximately 8-fold selectivity relative to COX-1.[35] The COX-2 enzyme has the primary role in prostaglandin production with respect to the inflammatory process. Inhibition of prostaglandin and cytokines in the acute inflammatory phase is theorized to decrease the vascular and cellular responses that lead to bone regeneration and healing.[19,36-38] Prostaglandins are known to promote angiogenesis[39] and have direct effects on increasing osteoblast cell division and osteoid production.[40,41] Tanaka and colleagues[42] also found that stimulation of prostaglandin E2 receptors accelerated bone repair and stimulated osteogenesis. Therefore, we would expect a more dramatic effect on fracture inhibition with a COX-2 inhibitor than with a nonselective NSAID. Simon and colleagues[22] showed that COX-2 has an essential function during normal fracture healing and that COX-2–selective NSAID inhibition of prostaglandin synthesis stops normal fracture healing. In addition, Robertson and colleagues[43] demonstrated that the COX-2 enzyme has an important regulatory role in bone homeostasis in mice.

Our observations support the hypothesis that inhibition of the COX-2 enzyme impairs fracture healing. We used a rat closed femur fracture model to show that biomechanical strength is markedly ($P = .0199$) decreased in animals given celecoxib. Mean force needed to break femurs in our treatment group (70.476 N) was significantly ($P = .02$) lower than that in our control group (91.190 N) when taken across all healing times. In addition, we found no statistical difference between these groups at the 4 sacrifice intervals (2, 4, 8, and 12 weeks). Directly measured callus did not differ significantly between the treatment and control groups—a result that may be related to an imperceptible sensitivity error in the calipers used. However, previous studies have had similar quantitative callus results.

Our raw data show that celecoxib had the most pronounced effect on the strength of fracture callus early in the inflammatory phase (2 weeks). During this phase, there was a 50% reduction in fracture strength in our treatment group compared with the control group. Brown and colleagues[44] found that rats treated with celecoxib had reduced stiffness, strength, radiographic evidence of healing, and histologic grade of callus 4 weeks after fracture. In their study, fractures were produced in the manner described by Bonnarens and Einhorn[45] (with use of a materials testing machine), and the drug was delivered with chocolate. We used a different

fracture method and a different drug-delivery method. Our results showed that impairment of fracture healing at 4 intervals was not statistically significant. The findings of Brown and colleagues are consistent with the data from our study.

The estimated plasma half-life of celecoxib after a single dose is 4 hours in male rats (vs 12 hours in humans).[46] Consequently, we thought that our rats' blood drug levels might be an underestimation of humans' COX-2 inhibition levels over a 24-hour period. Orally dosing rats every 4 hours for up to 12 weeks was felt to be an extremely difficult undertaking. Brown and colleagues[44] dosed their study animals with drug plus chocolate just once a day. In their scheme, blood drug levels would be high for only a very short time. Their dosing regimen ensures that rats receive the drug, but we believe that blood drug levels throughout most of the day would be negligible. Appropriate drug delivery remains an issue in fracture studies. The goal of providing laboratory animals with reasonable blood drug levels comparable to human levels, both in quantity and duration, has not been achieved in any fracture study. More work needs to be done to determine the true effects that a steady dose of anti-inflammatory medication has on fracture healing.

Our study rats received celecoxib daily until they were sacrificed. This scenario is unlike clinical scenarios in which COX-2 inhibitors are used acutely to manage early pain, inflammation, and swelling after a fracture. Investigators have thought that anti-inflammatory drugs, even those given daily only for a short time after injury, can impair the early inflammatory processes enough to affect bone formation and healing.[20,47] Gerstenfeld and colleagues[48] recently demonstrated that COX-2–specific drugs inhibit fracture healing more than nonspecific NSAIDs do and that the magnitude of this effect is related to treatment duration. After treatment was discontinued, however, prostaglandin E2 levels were gradually restored, and fracture callus strength returned to levels similar to those of controls.

In another recent study, Simon and O'Connor[49] demonstrated that higher doses and longer periods of celecoxib treatment were more detrimental to fracture healing than were lower doses and shorter periods. In contrast to Gerstenfeld and colleagues, Simon and O'Connor found that short-term use of a high-dose COX-2 inhibitor had a deleterious effect on ultimate fracture healing. Drug dosage levels may contribute to the discrepancy in these study results. We dosed our animals at approximately 3.2 mg/kg/d, similar to the 4-mg/kg/d dose used by Simon and O'Connor. Although we did not stop the drug during our study, we found a similar trend for continued impairment of fracture callus strength throughout the healing process in our treatment group compared with our control group. Some authors have recommended avoiding or discontinuing use of these medications during bone healing.[22,38,49,50] Clearly, continued research on fracture healing is needed so that clinicians can be appropriately advised regarding use of NSAIDs and COX-2 inhibitors during fracture healing.

Conclusions

This study was designed to examine the effects of COX-2 inhibitors on biomechanical strength and direct callus measurement in fracture healing. We found a statistically significant difference in the biomechanical strength of fracture callus in the pooled data from our treatment rats and control rats, but we did not find any statistically significant difference in biomechanical strength at 4 sacrifice intervals (2, 4, 8, and 12 weeks). At the 8-week interval, more callus was noted in our treatment group relative to the control group. However, no statistically significant difference was found in mean elliptical area of callus formation at any interval. Our data suggest that COX-2 enzyme function is important for fracture healing and that caution should be used when considering use of COX-2 inhibitors in patients with fractures.

Authors' Disclosure Statement

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