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BONE BIOLOGY

Short-term perioperative parecoxib is not detrimental to shaft fracture healing in a rat model

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Objectives

Experimental studies indicate that non-steroidal anti-inflammatory drugs (NSAIDs) may have negative effects on fracture healing. This study aimed to assess the effect of immediate and delayed short-term administration of clinically relevant parecoxib doses and timing on fracture healing using an established animal fracture model.

Methods

A standardized closed tibia shaft fracture was induced and stabilized by reamed intramedullary nailing in 66 Wistar rats. A 'parecoxib immediate' (Pi) group received parecoxib (3.2 mg/kg bodyweight twice per day) on days 0, 1, and 2. A 'parecoxib delayed' (Pd) group received the same dose of parecoxib on days 3, 4, and 5. A control group received saline only. Fracture healing was evaluated by biomechanical tests, histomorphometry, and dual-energy x-ray absorptiometry (DXA) at four weeks.

Results

For ultimate bending moment, the median ratio between fractured and non-fractured tibia was 0.61 (interquartile range (IQR) 0.45 to 0.82) in the Pi group, 0.44 (IQR 0.42 to 0.52) in the Pd group, and 0.50 (IQR 0.41 to 0.75) in the control group (n = 44; p = 0.068). There were no differences between the groups for stiffness, energy, deflection, callus diameter, DXA measurements (n = 64), histomorphometrically osteoid/bone ratio, or callus area (n = 20).

Conclusion

This study demonstrates no negative effect of immediate or delayed short-term administration of parecoxib on diaphyseal fracture healing in rats.

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Keywords: Fracture healing, Cyclooxygenase inhibitors, Biomechanical, Histology, Rats

Article focus

- Cyclooxygenase (COX) inhibitors are used against pain following fractures and surgery, but several animal studies suggest a negative effect on bone healing.
- A previous study from our group utilizing the same model showed that parecoxib for seven days (medium term) impaired fracture healing.
- Is short-term perioperative administration of parecoxib unfavourable for fracture healing?

Key messages

- Three days of immediate or delayed parecoxib administration did not compromise diaphyseal fracture healing in rats.
- The effect of parecoxib on fracture healing seems to be time-dependent.

Strengths and limitations

- We used a standardized fracture model and clinically relevant dose and duration of parecoxib.
- Results from animal studies may not be directly translatable to humans.

Introduction

Sufficient pain control following fracture surgery is important in order to facilitate early mobilization, limit the risk of postoperative complications, and promote fracture healing. In trauma patients with fractures, non-steroidal anti-inflammatory drugs (NSAIDs)

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are commonly used due to their analgesic, anti-inflammatory, and antipyretic effects. NSAIDs inhibit cyclooxygenase (COX), a key enzyme essential for upregulating cell signalling prostaglandins, which is crucial for musculoskeletal tissue healing. However, there are concerns about the negative impact of COX inhibitors on bone metabolism.[1-4] Previous experimental studies indicate that both selective and non-selective COX-2 inhibitors can have a negative effect on fracture healing.[5-8] The effects of COX-2 inhibitors on bone metabolism and fracture healing are yet to be fully understood, although COX-2 has been shown to be essential for both induction of osteoblastogenesis[9] and for fracture healing.[10]

Due to the putative adverse effect of COX inhibitors on fracture healing, there are safety concerns attached to the use of COX inhibitors in pain management following fracture surgery. To our knowledge, studies assessing time- and dose-dependent effects of COX inhibitors on musculoskeletal healing are sparse. Parecoxib, a potent, effective analgesic, anti-inflammatory, and opioid-sparing drug without platelet inhibition, is used clinically for pain management in orthopaedic surgery.[11] Previously, we have found that seven days of treatment with parecoxib delayed fracture healing in a rat model.[8] The aim of the current study was to investigate the effect of immediate and delayed short-term treatment (three days) with clinically relevant parecoxib doses on fracture healing in an established closed tibia shaft fracture model in rats.

Materials and Methods

Replacement, refinement, and reduction were all taken into serious consideration when planning the study. Humane endpoints were defined in order to ensure animal welfare throughout the study.

Animals. Wistar rats (n = 66) (Taconic Europe, Lille Skensved, Denmark), skeletally mature with a mean weight of 288 g (265 to 330), were included in the study. Due to an increased metabolism of COX inhibitors in male compared with female rats,[12,13] our previous studies utilizing the same model were conducted in female rats only.[7,8] Female rats were chosen for the current study to ensure internal quality control.

The animals were kept in pairs, each pair in a wire-topped plastic cage in an accredited animal facility in a controlled environment (mean temperature 21°C (SD 1°C), humidity 55% (SD 10%), ventilation air volume exchanged 20 times per hour, alternating 12-hour light and dark cycles). They were allowed free access to water and standard laboratory rodent nutrition (MR3, SD'S, Essex, United Kingdom, with 1.24% total calcium, 0.83% phosphorus, and 2948 IU/kg vitamin D3). Following ten days of acclimatization, all animals underwent the surgical procedure with general anaesthesia induced and maintained by Hypnorm (fluanisone 5 mg/ml and fentanyl citrate 0.1575 mg/ml; Jansen Pharmaceutica BV, Beerse, Belgium) and Dormicum (midazolam 2.5 mg/ml; Hoffmann La Roche, Basel, Switzerland) diluted in an equal amount of sterile water. The dose of this working solution was 0.2 ml/100 g body weight administered subcutaneously for anaesthesia. Antibiotics were not used. Inhalation anaesthesia (1% to 2.5% isoflurane; Isocare vet, Animalcare, York, United Kingdom) was used for the dual-energy x-ray absorptiometry (DXA) at two weeks. To ensure sufficient postoperative pain control, a subcutaneous dose of 0.05 mg/kg Buprenorphine (0.3 mg/ml) (Temgesic; Schering–Plough, Kenilworth, New Jersey) was administered postoperatively in all animals, and then every 12 hours for the first two days. Animal behaviour, appearance, movement, body weight, and wound status were registered throughout the study period in a predefined form with scores, defining thresholds for extra administration of buprenorphine and humane endpoints.

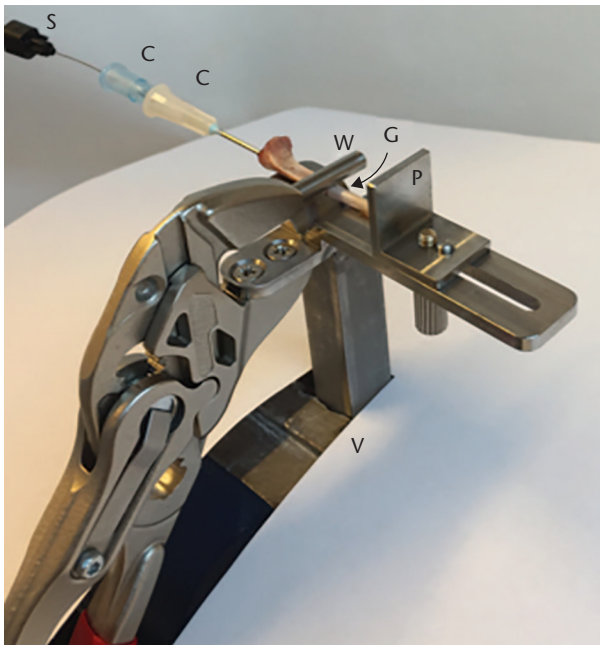

Fig. 1

Three-point loading fracture forceps secured in a vice (V). Photo from the pilot study using bone without soft tissue, showing the components of the nail: the stylus (S); and the two cannulas (C). *In vivo*, the heel was pressed against an adjustable plate (P) in order to obtain a standardized midshaft fracture as the wedge (W) pressed the leg into the groove (G) when the forceps was clamped.

Surgical procedure. A 3 mm skin incision was made laterally on the right thigh 15 mm proximal to the knee to prevent conflict between the nail and wound. The skin was retracted distally to expose the patellar tendon. The nail consisted of two cannulas (23G 0.6 × 60 mm Sterican; Braun GmbH, Kronberg, Germany; 19G 1.1 × 40 mm Microlance 3; BD, Madrid, Spain), in addition to a spinal needle stylet (22G 0.7 × 90 mm Yale Spinal; BD). This was introduced to the medullary canal of the tibia medial to the patellar tendon. The canal was reamed using the

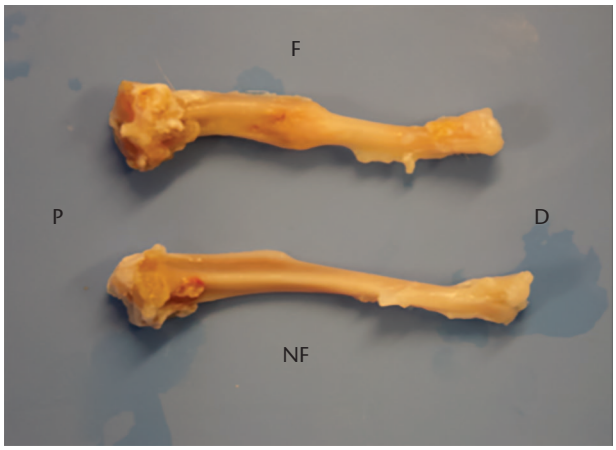

Fig. 2a

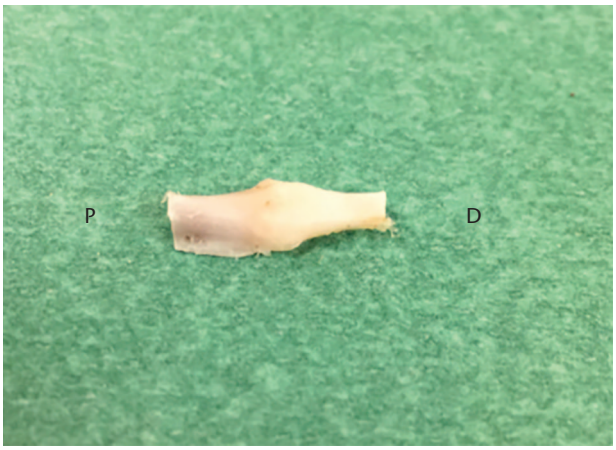

Fig. 2b

Lateral-view photographs of tibia *ex vivo* at four weeks of healing. Orientation is proximal (P) to distal (D). a) Fractured (F) and non-fractured (NF) tibia from a specimen ('parecoxib immediate' (Pi) group) prepared for biomechanical tests. b) Segment of middle tibia containing callus in a specimen (control group) fixed for histological analysis.

cannulas as they were advanced toward the distal tibia. The two cannulas were withdrawn to the proximal tibia after sufficient reaming of the canal. A standardized closed midshaft tibia fracture was created with the stylet remaining inside the full length of the medullary canal as a guidewire. This enabled technically easy fracture reduction as the two cannulas were reintroduced over the fracture site. Finally, the nail was cut flush with the tibial plateau before the skin was closed with absorbable sutures. Unrestricted weight-bearing was allowed for all rats.

The custom-made three-point fracture-inducing forceps were a modified version of previously described forceps.[14] The modified forceps' ability to induce a closed standardized midshaft tibial fracture, with minimal soft-tissue damage, was assessed in a pilot study on cadaveric rat tibiae with and without soft tissue prior to this experiment (Fig. 1).

Study groups. Single animals were allocated by computerized randomization into two parecoxib intervention groups or a control group (i.e. 22 animals in each group). The study staff was blinded to the group and outcome allocation.

All animals received injections (parecoxib or saline, volume 0.04 ml intramuscular) twice a day for six days perioperatively; a total of 12 injections. The daily dose of parecoxib (Dynastat; Pfizer, Puurs, Belgium) was estimated to be 6.4 mg/kg body weight, using allometric scaling based on the basic calorific demand, which is increased by a factor of four in rats compared with humans.[15-17] The 'parecoxib immediate' (Pi) group received the first dose of parecoxib 30 minutes preoperatively, then on days 0 to 2 postoperatively, and then saline injections on days 3 to 5. The 'parecoxib delayed' (Pd) group received saline injections on days 0 to 2, then parecoxib injections on days 3 to 5. The control (C) group received saline injections on days 0 to 5, and no COX inhibitors. All animals were killed at four weeks.

Biomechanical testing. Animals that were allocated to biomechanical tests were killed by a pentobarbital overdose (Pentobarbital-Natrium vet, 100 mg/ml; Norsk Medicinaldepot, Oslo, Norway) under general anaesthesia. The harvested tissue was preserved in Ringer Acetat (Fresenius Kabi, Oslo, Norway) at –20°C until testing. Following thawing, both tibiae were dissected free of soft tissue, and the fibulas were removed. The anteroposterior (AP) and mediolateral (ML) diameters of callus formation in the right tibiae were measured using a sliding caliper with an accuracy of ± 0.01 mm. The nail was carefully removed and fracture union was evaluated macroscopically (Fig. 2a). The tibiae were loaded in a three-point ventral cantilever bending test to failure,[8,18] using a hydraulic material testing machine (Model 858 Mini Bionix with MTS FlexTest digital controller; MTS Systems Corporation, Eden Prairie, Minnesota). A preload of 0.5 N was applied prior to the start of the test to remove any lag from the setup. With a 250 N load cell, speed set to 2.67 mm per second, yielding a tibia bending rate of 7.2° per second, with the fulcrum placed over the fracture callus, data were collected at 20 Hz. The non-fractured left tibia was fractured at the same level in the same manner. This allowed for the collection of data on force, time, and deflection, which were exported to Origin (Origin v. 8.6 for Windows; OriginLab Corporation, Northampton, Massachusetts) for final analysis of ultimate bending moment, stiffness, energy absorption, and deflection.

Histological evaluation. Tissues of animals allocated to histological evaluation were fixed *in vivo* by vascular perfusion of 0.1 M phosphate-buffered 2% paraformaldehyde during deep anaesthesia. Following removal of the nail, fibula, and soft tissues, a 15 mm tibial shaft segment containing the fracture with callus was harvested using an oscillating saw (Fig. 2b). These segments were fixed overnight in the same fixative as mentioned above, dehydrated in series of ethanol, incubated, and embedded in

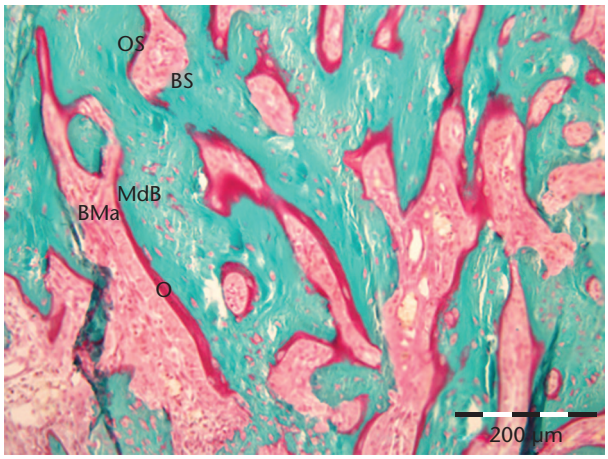

Fig. 3a

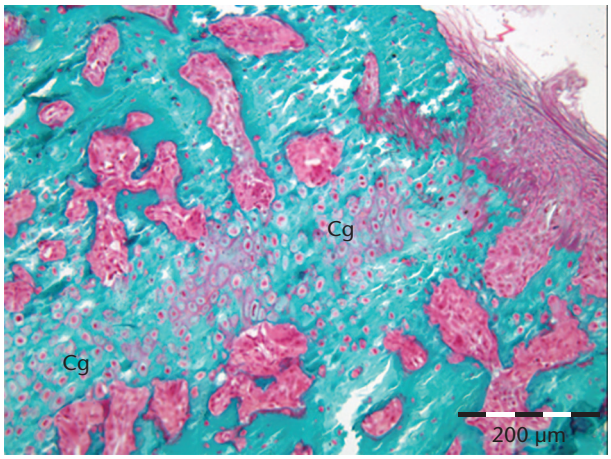

Fig. 3b

High-power magnification light microscopy of tibial fracture calluses. Masson–Goldner trichrome staining of mineralized tissue provides good differentiation between osteoid (O, purple) and mineralized bone matrix (MdB, green). Non-mineralized bone marrow (BMa, red) is distributed between the mineralized bone trabeculae. a) Woven bone formation (control group) with mainly osteoid trabecular surfaces (OS) and a few bone surfaces (BS). b) An immature region within the callus-containing cartilage (Cg) and partly mineralized matrix with ongoing endochondral ossification. Scale bars = 200 µm.

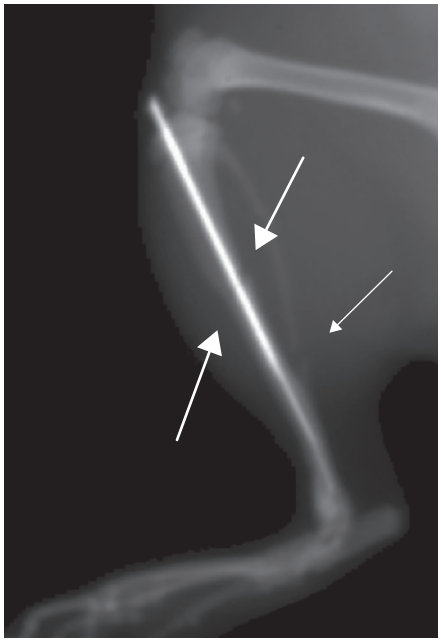

Fig. 4

Representative lateral radiograph at baseline showing a short oblique tibial fracture (large arrows) fixed with an intramedullary nail and concomitant fibular fracture (small arrow). Specimen from the control group.

plastic (K-plast; DiaTec Systems for Laboratory, Hallstadt, Germany), following a standard protocol. The undecalcified specimens were cut from anterior to posterior (thickness 5 µm). The central section containing the thickest callus was chosen as the region of interest (ROI).

Histomorphometry. Masson–Goldner's trichrome-stained sections were evaluated by light microscopy. Digital images were analyzed using AnalySIS V (Olympus Soft Imaging Solutions, Münster, Germany). The ROI was defined at 1.25 × magnification by outlining the perimeter of the callus, including any cortical fracture surface, but excluding original cortical or trabecular bone, periosteum, and bone marrow. This ROI was defined as total callus area. A virtual grid of lines (random angles, space 100 µm) was superimposed onto sections at 10 × magnification. Presence of osteoid surfaces or mineralized bone surfaces was noted. The osteoid surface per bone surface (OS/BS; %) were calculated as an indirect measure of bone formation (Fig. 3a). Presence of cartilage within the callus ROI was registered in each specimen (Fig. 3b). The nomenclature used is in accordance with the recommendations of the American Society for Bone and Mineral Research (ASBMR).[19]

Bone mineral measurement and radiological evaluation. Bone mineral density (BMD) and bone mineral content (BMC) were measured over the fracture site in all animals *in vivo* using DXA (Lunar PIXImus with software v. 2.10; Lunar, Madison, Wisconsin) immediately after surgery, and again at two and four weeks. The ROI (14 × 14 pixels) was aligned over the fracture and included the anterior cortical bone, the callus, and the nail. The BMD and BMC values were corrected for an independently measured value for the nail before final analysis. Radiographs were reviewed for fracture pattern and potential complications (Fig. 4).

Statistical analysis. An *a priori* power calculation for the sample size was not performed, but the number of animals was estimated based on previous studies.[7,8] The main outcome was the ultimate bending moment ratio between fractured and non-fractured tibia. Other outcomes were ratios of bending stiffness, energy absorption, deflection, callus AP and ML diameter, and time-dependent difference in BMD and BMC. Two independent observers (GAH and SN for biomechanical variables; GAH and ES for DXA variables) analyzed these outcomes, and the mean values were calculated and used for the statistical analyses. Normality of the distribution was evaluated using

Table I. Biomechanical results showing ratios between fractured and non-fractured tibia and callus diameters at four weeks

Group	Parecoxib immediate (n = 15)	Parecoxib delayed (n = 15)	Control (n =14)	p-value*
Median moment, ratio (IQR)	0.61 (0.45 to 0.82)	0.44 (0.42 to 0.52)	0.50 (0.41 to 0.75)	0.068
Median stiffness, ratio (IQR)	1.07 (0.85 to 1.46)	1.01 (0.89 to 1.13)	1.22 (0.75 to 1.37)	0.691
Median energy, ratio (IQR)	0.39 (0.21 to 0.5)	0.22 (0.17 to 0.3)	0.26 (0.19 to 0.39)	0.127
Median deflection, ratio (IQR)	0.69 (0.54 to 0.84)	0.55 (0.46 to 0.61)	0.54 (0.45 to 0.78)	0.083
Median AP diameter, mm (IQR)	4.5 (3.9 to 5.1)	5.1 (4.2 to 5.9)	4.4 (3.7 to 4.9)	0.082
Median ML diameter, mm (IQR)	3.1 (2.8 to 3.8)	3.5 (2.9 to 3.8)	3.2 (2.9 to 3.5)	0.590

*Kruskal–Wallis test
IQR, interquartile range; AP, anteroposterior; ML, mediolateral

Table II. Difference in bone mineral density (BMD) and bone mineral content (BMC) from baseline

Group	Parecoxib immediate (n = 22)	Parecoxib delayed (n = 22)	Control (n = 20)	p-value*
Mean BMD: 2 wks, mg/cm^2 (range)	101 (-32 to 282)	58 (-161 to 224)	75 (-56 to 182)	0.171
Mean BMC: 2 wks, mg (range)	7 (1 to 16)	4 (-9 to 17)	6 (1 to 13)	0.153
Mean BMD: 4 wks, mg/cm^2 (range)	152 (15 to 281)	106 (-69 to 341)	103 (-11 to 210)	0.106
Mean BMC: 4 wks, mg (range)	10 (1 to 20)	8 (-7 to 22)	7 (1 to 15)	0.257

*One-way analysis of variance (ANOVA) test

histograms with normality curves. Homogeneity of variance for the main outcome was proven by Levene's test (p = 0.073). Biomechanical and histomorphometrical data are presented as medians and interquartile ranges (IQRs), and groups are compared using the Kruskal–Wallis test. A non-parametric test was used due to limited sample size. The BMD and BMC differences are presented as mean and range, and the groups compared with one-way analysis of variance (ANOVA). The alpha level was set to 0.05. Statistical analyses were performed using IBM SPSS Statistics for Macintosh v. 24.0 (IBM, Armonk, New York).

Results

Animal inclusion. The body weight of all animals was measured every week throughout the study period. At four weeks, the mean increase in body weight from baseline was 13.4 g (-15 to 25), with no difference between the study groups (p = 0.510, one-way ANOVA). A closed midshaft fracture was successfully created in all animals (n = 66), and correct placement of the nail to stabilize the fracture was confirmed in radiographs postoperatively. Four animals had minor wound problems that were resolved without any further issues. No animals were excluded due to infection or penetration of the skin by the nail. Two animals (control group) died during anaesthesia, leaving a total of 64 animals available for final analyses. Next, 44 animals (14 or 15 from each of the three study groups) were assessed biomechanically. The remaining 20 animals (six or seven from each study group) were assessed histologically.

Biomechanical assessment. No nonunion was observed. A few degrees of external rotation were observed in most of the fractures. Overall, the median maximum bending moment of fractured tibiae was 18.9 Nm × 10^{-2} (IQR 14.9 to 22.4) compared with 35.1 Nm × 10^{-2} (IQR 30 to 38.9) in non-fractured tibiae. Ratios of fractured and non-fractured tibia in ultimate bending moment, stiffness, energy, and deflection showed no differences between the study groups (Table I). The macroscopically measured callus diameters were also equal between the groups.

Bone mineral measurements and radiology. Overall, mean BMD at baseline was 160 mg/cm^2 (3 to 267), 240 mg/cm^2 (67 to 357) at two weeks, and 280 mg/cm^2 (146 to 474) at four weeks. There were no differences between the groups in BMD or BMC from baseline to two or four weeks (Table II). The baseline radiographs showed that all fractures were simple transverse or short oblique fractures. Wedge fragments were observed in only 2/64 fractures (3%), and no comminuted fractures were observed. The fibula was fractured 2 mm to 5 mm proximal to the rat anatomical tibiofibular synostosis in 44/64 specimens (69%). Radiographs obtained at four weeks demonstrated fracture healing by callus formation in all animals. Nail migration was not observed in any of the specimens.

Histological findings. Light microscopy confirmed the radiological evaluation of fracture patterns. All fractures were healed, as defined by a mineralized callus with woven bone formation bridging the cortical fracture gap at two sides (Fig. 5). Two fractures (Pi and Pd groups) had a bridging callus on the compression side and fibrous tissue in the fracture gap of the tension side. A bridging callus was observed on both sides in the coronal sections in 18/20 fractures (90%). Hyaline cartilage with vascular invasion suggested that early endochondral ossification was still taking place (Fig. 3b). It was observed in the calluses of nine fractures and evenly distributed among the groups (Table III). The remaining 11 had more mature calluses, consisting mainly of woven bone with both non-mineralized (osteoid) and mineralized bone matrix (Fig. 6). The OS/BS (%), as measured by

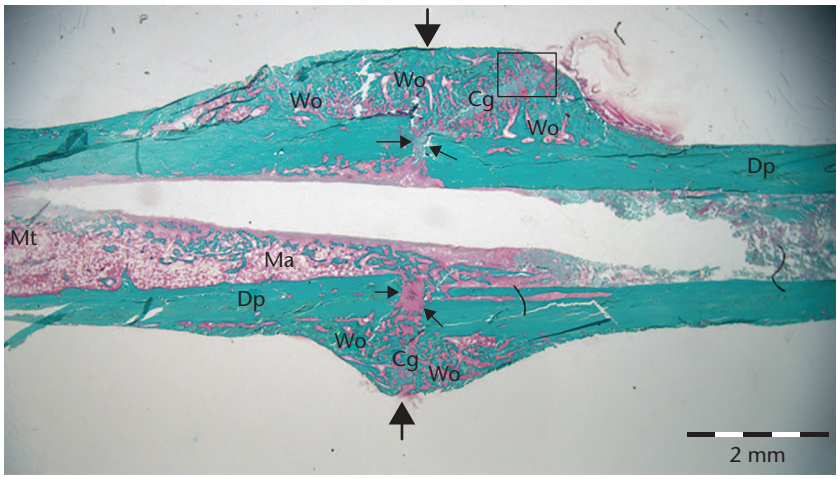

Fig. 5

Low-power magnification light microscopy of Masson–Goldner trichrome-stained coronal section of diaphyseal (Dp) tibial fracture (between large arrows) at four weeks ('parecoxib immediate' (Pi) group), showing bridging callus formation on both sides. Callus consists of woven bone (Wo) and immature cartilage (Cg). The metaphyseal (Mt) region in the section is localized proximally. Small arrows indicate the original cortical fracture gap. The medullary canal contains displaced bone marrow (Ma). The clear space represents the area of the extracted nail. The rectangle shows the area displayed at high magnification in Figure 3b. Scale bar = 2 mm.

Table III. Histomorphometric measurements of osteoid surfaces per bone surface (OS/BS; %), total callus area, and presence of immature cartilaginous zones

Group	Parecoxib immediate (n = 7)	Parecoxib delayed (n = 7)	Control (n = 6)	p-value*
Median OS/BS, % (IQR)	*38* (30 to 47)	*48* (39 to 53)	*49* (44 to 52)	0.092
Median callus area, mm^2 (IQR)	13.5 (11.7 to 18.7)	11.3 (8.3 to 14.5)	11.6 (7.5 to 19.8)	0.282
Cartilaginous zones, n	3	4	2	0.697

*Kruskal–Wallis test
IQR, interquartile range

histomorphometry, was equally distributed between the groups, and no difference in total callus area was noted (Table III).

Discussion

This study shows that immediate or delayed short-term treatment with parecoxib did not compromise closed tibia shaft fracture healing at four weeks. The parecoxib dose used in rat studies varies. We used a relatively high daily dose of 6.4 mg/kg body weight, as suggested by Virchenko et al,[20] to prevent the risk of type II experimental error. In previous studies, utilizing the same model, we found that seven days of parecoxib treatment delayed early fracture healing. Even with a daily parecoxib dose as low as 1 mg/kg body weight, bending moment, stiffness, and BMD were significantly reduced at three weeks.[7,8] In the current study, immediate or delayed three-day treatment with parecoxib did not negatively affect the biomechanical properties, callus maturation, or bone formation at four weeks, despite using a 2.7-fold larger dosage in this study than in our previous studies. In light of the previous findings of a negative impact with the seven-day parecoxib treatment, the fact that the current study showed no effect at three days of treatment may provide useful additional information. Thus, our collective findings could be indicative of a time-dependent effect of parecoxib on fracture healing. The COX-2 messenger RNA (mRNA) level remained elevated for two weeks after femoral fracture in a rat study by Gerstenfeld et al.[21] In a mouse study, the COX-2 mRNA level peaked four days following a muscle injury, but the maximum COX-2 protein concentration was observed as early as day 1.[22] Delayed short-term perioperative administration of COX inhibitors may be a plausible strategy to avoid interference with fracture healing, but a three-day delay of a three-day treatment period did not compromise fracture healing in our study. However, due to interspecies differences in fracture healing and drug metabolism, the translational potential is uncertain and should be taken into consideration when interpreting our findings.

The tibial shaft fracture model in rats is well established and has recently been further developed by our group. As opposed to osteotomy models, the closed fracture model may have an advantage in validity. Moreover, the model allowed reaming of the medullary canal, similar fracture patterns, reliable reduction, and fixation of the fracture. Refinements, compared with our previous fracture studies, included blinding of the study staff, randomized allocation of animals to limit any bias, and two independent observers analyzing biomechanical and

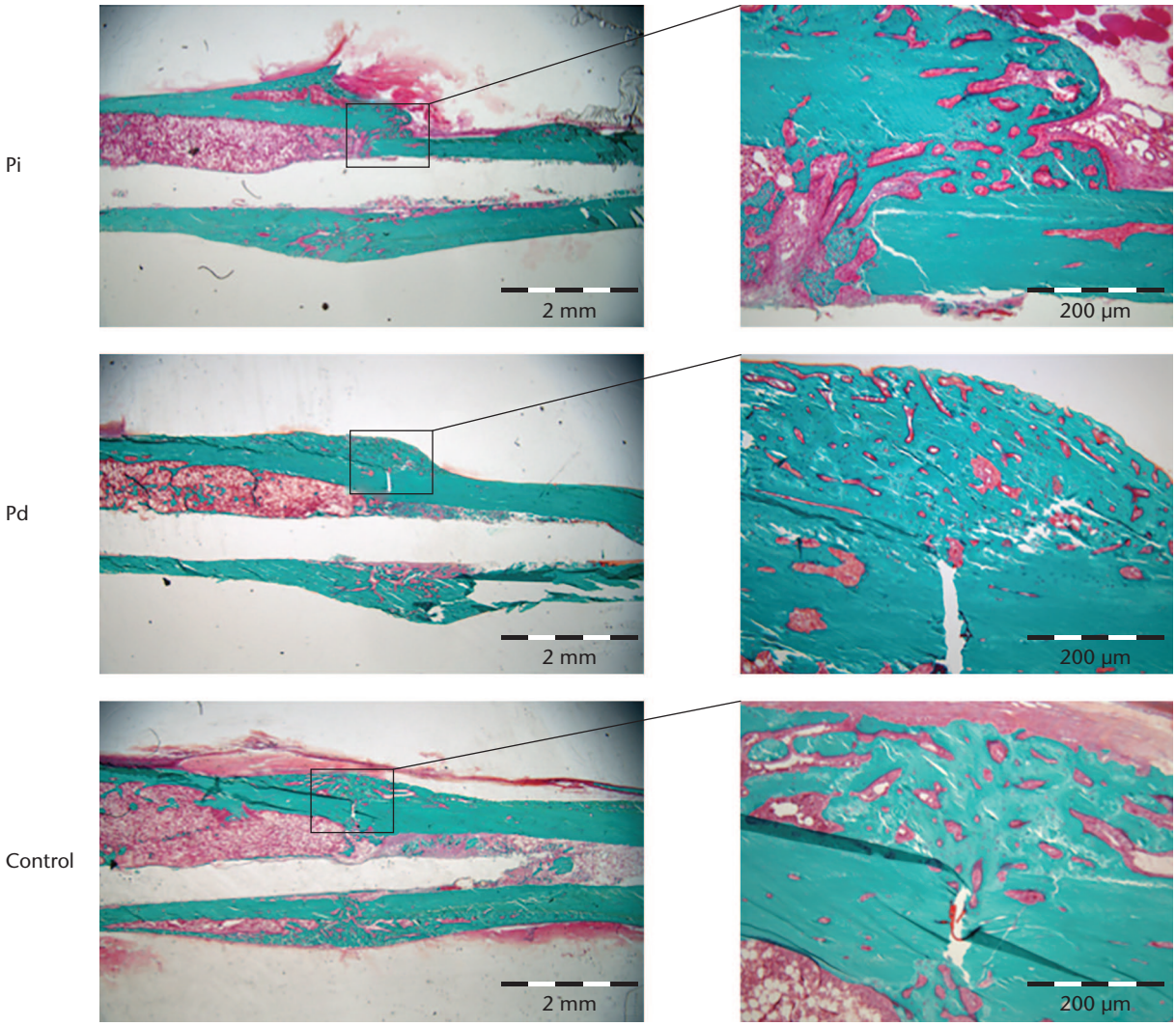

Fig. 6

Low-power (left panels) and high-power (right panels) magnification light microscopy of representative Masson–Goldner trichrome-stained sections of tibial fractures from the three study groups. Qualitatively, no differences between the study groups were observed. Scale bars = 2 mm (left panels) and 200 µm (right panels).

bone mineral data. We also included fracture evaluation by histology.

Limitations of the three-point cantilever bending test and the BMD measurement that included the nail have been discussed in detail previously.[8] In addition, the method of fracture induction with a concomitant fibular fracture and fracture fixation without blocking of rotation could explain the observed tendency of fracture healing in external rotation in most specimens. However, all fractures healed without any further complications, and a normal pattern of secondary bone healing was observed by histology. Therefore, we believe the nail provided sufficient stability to evaluate the aim of this study. However, the results may not be directly translated to a clinical setting, where the instability from the concomitant fibular fracture is compensated for by interlocking of the tibial nail.

Delayed shaft fracture union is reported in several animal studies with COX-2 inhibitors, including rofecoxib,[23] celecoxib,[5] and parecoxib.[24] COX-2 inhibition may adversely affect healing, particularly in the early phase following the fracture.[6,25] However, if the duration of treatment is short, the negative biological effect seems to be reversible,[24] which could explain the findings in the present study.

The negative effect of COX inhibition on fracture healing seems well documented in experimental studies.[26] However, these negative effects have been hard to reproduce in clinical trials, as they depend on dose, drug metabolism, timing and duration of treatment, soft-tissue damage, and fracture pattern. Indomethacin (non-selective COX inhibitor) greatly impaired healing of diaphyseal fractures, but had only minimal effect on metaphyseal fractures in mice.[27] Animal studies often

utilize cortical fracture models while patients more often have corticocancellous fractures. Differences in fracture location and stability may therefore explain some of the discrepancies between experimental studies and clinical experience. In addition, other regulatory pathways, including transforming growth factor beta and Wnt, and epigenetic regulators are involved in bone metabolism and fracture healing, which further complicates the overall understanding.[28,29] Also, a variety of patient-related confounding factors are likely to influence bone healing.

The number of clinical studies of COX inhibitors in humans is limited. Long-term (six weeks) treatment with indomethacin to prevent heterotopic ossification in trauma patients with acetabular fractures revealed an increased risk for development of nonunion in concomitant long bone fractures.[30] Giannoudis et al[31] also reported an association between COX inhibitors and increased risk of nonunion in femoral fractures in a retrospective study. The correlation between COX inhibitors and nonunion was especially strong when COX inhibitors were used for longer than four weeks. In addition, medium-term use (one week) of COX inhibitors was associated with a prolonged time to union in this study. A systematic review concluded that short-duration COX inhibitor administration is safe concerning bone healing,[32] while another stated that COX-2 inhibitors should be considered a potential risk factor for fracture healing, and should therefore be avoided in patients at risk of delayed fracture healing.[33] The current understanding of the overall effect of COX inhibitors on bone healing remains limited[34] and lacks the clinical evidence to support strong recommendations.[35]

In conclusion, this study demonstrated no negative effect of immediate or delayed three-day treatment with parecoxib on diaphyseal fracture healing in rats.

References

1. **Aspenberg P.** Don't administer NSAID after bone surgery! *Lakartidningen* 2002;99:2554. (Article in Swedish)
2. **Aspenberg P.** Drugs and fracture repair. *Acta Orthop* 2005;76:741-748.
3. **Su B, O'Connor JP.** NSAID therapy effects on healing of bone, tendon, and the enthesis. *J Appl Physiol (1985)* 2013;115:892-899.
4. **Barry S.** Non-steroidal anti-inflammatory drugs inhibit bone healing: a review. *Vet Comp Orthop Traumatol* 2010;23:385-392.
5. **Bergenstock M, Min W, Simon AM, Sabatino C, O'Connor JP.** A comparison between the effects of acetaminophen and celecoxib on bone fracture healing in rats. *J Orthop Trauma* 2005;19:717-723.
6. **Simon AM, O'Connor JP.** Dose and time-dependent effects of cyclooxygenase-2 inhibition on fracture-healing. *J Bone Joint Surg [Am]* 2007;89-A:500-511.
7. **Dimmen S, Nordsletten L, Engebretsen L, Steen H, Madsen JE.** Negative effect of parecoxib on bone mineral during fracture healing in rats. *Acta Orthop* 2008;79:438-444.
8. **Dimmen S, Nordsletten L, Madsen JE.** Parecoxib and indomethacin delay early fracture healing: a study in rats. *Clin Orthop Relat Res* 2009;467:1992-1999.
9. **Zhang X, Schwarz EM, Young DA, et al.** Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. *J Clin Invest* 2002;109:1405-1415.
10. **Simon AM, Manigrasso MB, O'Connor JP.** Cyclo-oxygenase 2 function is essential for bone fracture healing. *J Bone Miner Res* 2002;17:963-976.
11. **Diaz-Borjon E, Torres-Gomez A, Essex MN, et al.** Parecoxib provides analgesic and opioid-sparing effects following major orthopedic surgery: a subset analysis of a randomized, placebo-controlled clinical trial. *Pain Ther* 2017;6:61-72.
12. **Halpin RA, Geer LA, Zhang KE, et al.** The absorption, distribution, metabolism and excretion of rofecoxib, a potent and selective cyclooxygenase-2 inhibitor, in rats and dogs. *Drug Metab Dispos* 2000;28:1244-1254.
13. **Paulson SK, Zhang JY, Breau AP, et al.** Pharmacokinetics, tissue distribution, metabolism, and excretion of celecoxib in rats. *Drug Metab Dispos* 2000;28:514-521.
14. **Ekeland A, Engesaeter LB, Langeland N.** Mechanical properties of fractured and intact rat femora evaluated by bending, torsional and tensile tests. *Acta Orthop Scand* 1981;52:605-613.
15. **West GB, Brown JH, Enquist BJ.** A general model for the origin of allometric scaling laws in biology. *Science* 1997;276:122-126.
16. **Schneider K, Oltmanns J, Hassauer M.** Allometric principles for interspecies extrapolation in toxicological risk assessment—empirical investigations. *Regul Toxicol Pharmacol* 2004;39:334-347.
17. **Huang Q, Riviere JE.** The application of allometric scaling principles to predict pharmacokinetic parameters across species. *Expert Opin Drug Metab Toxicol* 2014;10:1241-1253.
18. **Engesaeter LB, Ekeland A, Langeland N.** Methods for testing the mechanical properties of the rat femur. *Acta Orthop Scand* 1978;49:512-518.
19. **Dempster DW, Compston JE, Drezner MK, et al.** Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 2013;28:2-17.
20. **Virchenko O, Skoglund B, Aspenberg P.** Parecoxib impairs early tendon repair but improves later remodeling. *Am J Sports Med* 2004;32:1743-1747.
21. **Gerstenfeld LC, Thiede M, Seibert K, et al.** Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs. *J Orthop Res* 2003;21:670-675.
22. **Bondesen BA, Mills ST, Kegley KM, Pavlath GK.** The COX-2 pathway is essential during early stages of skeletal muscle regeneration. *Am J Physiol Cell Physiol* 2004;287:C475-C483.
23. **Goodman S, Ma T, Trindade M, et al.** COX-2 selective NSAID decreases bone ingrowth in vivo. *J Orthop Res* 2002;20:1164-1169.
24. **Gerstenfeld LC, Al-Ghawas M, Alkhiary YM, et al.** Selective and nonselective cyclooxygenase-2 inhibitors and experimental fracture-healing. Reversibility of effects after short-term treatment. *J Bone Joint Surg [Am]* 2007;89-A:114-125.
25. **Meunier A, Aspenberg P.** Parecoxib impairs early metaphyseal bone healing in rats. *Arch Orthop Trauma Surg* 2006;126:433-436.
26. **Cottrell J, O'Connor JP.** Effect of non-steroidal anti-inflammatory drugs on bone healing. *Pharmaceuticals (Basel)* 2010;3:1668-1693.
27. **Sandberg O, Aspenberg P.** Different effects of indomethacin on healing of shaft and metaphyseal fractures. *Acta Orthop* 2015;86:243-247.
28. **Walters G, Pountos I, Giannoudis PV.** The cytokines and micro-environment of fracture haematoma: current evidence. *J Tissue Eng Regen Med* 2018;12:e1662-e1677.
29. **Morcos MW, Al-Jallad H, Li J, et al.** PHOSPHO1 is essential for normal bone fracture healing: an animal study. *Bone Joint Res* 2018;7:397-405.
30. **Burd TA, Hughes MS, Anglen JO.** Heterotopic ossification prophylaxis with indomethacin increases the risk of long-bone nonunion. *J Bone Joint Surg [Br]* 2003;85-B:700-705.
31. **Giannoudis PV, MacDonald DA, Matthews SJ, et al.** Nonunion of the femoral diaphysis. The influence of reaming and non-steroidal anti-inflammatory drugs. *J Bone Joint Surg [Br]* 2000;82-B:655-658.
32. **Kurmis AP, Kurmis TP, O'Brien JX, Dalén T.** The effect of nonsteroidal anti-inflammatory drug administration on acute phase fracture-healing: a review. *J Bone Joint Surg [Am]* 2012;94-A:815-823.
33. **Geusens P, Emans PJ, de Jong JJ, van den Bergh J.** NSAIDs and fracture healing. *Curr Opin Rheumatol* 2013;25:524-531.
34. **Marquez-Lara A, Hutchinson ID, Nuñez F Jr, Smith TL, Miller AN.** Nonsteroidal anti-inflammatory drugs and bone-healing: a systematic review of research quality. *JBJS Rev* 2016;4:4.
35. **Richards CJ, Graf KW Jr, Mashru RP.** The effect of opioids, alcohol, and nonsteroidal anti-inflammatory drugs on fracture union. *Orthop Clin North Am* 2017;48:433-443.

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Author contributions

- G. A. Hjorthaug: Designed the study, Performed the surgeries, DXA measurements, histological analyses, and statistical calculations, Carried out the independent DXA analyses and biomechanical testing, Wrote the manuscript.
- E. Søreide: Designed the study, Performed the surgeries and DXA measurements, Carried out the independent DXA analyses and biomechanical testing.
- L. Nordsletten: Designed the study.
- J. E. Madsen: Designed the study.
- F. P. Reinholt: Designed the study, Supervised the histological analyses.
- S. Niratisairak: Performed the independent biomechanical analyses, Assisted in performing the biomechanical calculations.
- S. Dimmen: Designed the study, Performed the surgeries.

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