Negative effect of parecoxib on bone mineral during fracture healing in rats

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Background and purpose **Non-steroidal anti-inflammatory drugs (NSAIDs) are conventional cyclooxygenase (cox) inhibitors commonly used in musculoskeletal trauma to reduce the inflammatory response and pain, but they also seem to affect bone metabolism. Parecoxib is a cox inhibitor that selectively inhibits cox-2. Through their selective mechanism of action, these newer drugs are supposed to reduce the gastrointestinal side effects of conventional cox inhibitors. The effects on bone metabolism and healing have, however, not been fully elucidated. Thus, there are reasons for concern regarding the potential negative effects of these drugs on bone metabolism and bone repair. We investigated the effects of short-term administration of parecoxib on bone mineral formation and bone healing in rats.**

Animals and methods **26 female Wistar rats were given parecoxib intraperitoneally for 7 days after a closed tibial fracture that was stabilized with an intramedullary nail, and 26 animals were given saline. At 2, 3, and 6 weeks after surgery bone mineral density (BMD) at the fracture site was measured using dual-energy X-ray absorptiometry (DEXA). 6 weeks after the fracture, 14 rats from the parecoxib group and 16 rats from the placebo group were killed for mechanical testing, and the rest of the animals were killed for tissue analysis. The healing fractures and the intact contralateral tibias were mechanically tested by three-point cantilever bending.**

Results **The BMD at the fracture site was calculated as the average of the results after 2, 3, and 6 weeks. Mean BMD was lower in the parecoxib group, 0.23 (SD 0.06) g/cm^2, than in the control group, 0.27 (SD 0.05) g/cm^2 ($p = 0.01$). There were no statistically significant differences in mechanical properties of the healing fractures after 6 weeks. However, the study may have lacked sufficient statistical power to determine whether a negative effect on healing had occurred.**

Interpretation **No mechanical differences were detected between the control and treatment groups after 6 weeks, but they may have been present earlier in the fracture healing process. Our findings do, however, indicate that parecoxib given postoperatively for a week has a negative effect on mineralization during the early phase of fracture healing.**

Non-steroidal anti-inflammatory drugs (NSAIDs), which are conventional cyclooxygenase (cox) inhibitors, have been used to reduce postoperative inflammation and pain in orthopedic trauma and surgery during the past two decades. Several studies have proven their superior effects compared to other painkillers such as acetaminophen, codeine, and opioids (McLoughlin et al. 1990, Fogarty et al. 1995, Dahl et al. 2004).

There have, however, been concerns in using cox inhibitors in orthopedic fracture treatment due to possible negative effects on fracture healing (Aspenberg 2002, Einhorn 2002, Dahners and Mullis 2004, Simon and O'Connor 2007), as several studies have shown that conventional NSAIDs affect bone metabolism and delay fracture healing (Rø et al. 1976, Sudmann et al. 1979, Keller et al. 1989, Engesaeter et al. 1992, Hogevold et al. 1992, Beck et al. 2003).

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The analgesic and anti-inflammatory effects of conventional cox inhibitors have been attributed to the inhibition of cox-2, which is involved in the induction of pain and inflammation (Seibert et al. 1994, Dubois et al. 1998). During the past years, the cox-2 inhibitors have been introduced as the new anti-inflammatory drugs of choice for perioperative use. Compared to conventional cox inhibitors, the selective cox-2 inhibitors do not impair platelet function and they may therefore be safer regarding perioperative bleeding (Leese et al. 2000, Weber et al. 2003). It has been shown that, given preoperatively, cox-2 inhibitors lead to an efficient reduction in postoperative pain (Desjardins et al. 2001, Gan et al. 2004).

Several studies have compared ketorolac—the standard non-selective cox inhibitors for parenteral postoperative pain management (Gillies et al. 1987)—to parecoxib, a potent selective inhibitor of cox-2 (Talley et al. 2000). Due to the lack of anti-platelet effects, parecoxib has the potential of becoming the anti-inflammatory drug of choice for parenteral treatment of postoperative pain in patients with no previous history of cardiovascular problems (Stichtenoth and Frolich 2003, Kranke et al. 2004), and parecoxib compares favorably with ketorolac, acetaminophen, and opoids as a postoperative analgesic (Rasmussen et al. 2002, Hubbard et al. 2003, Malan et al. 2003).

It has been demonstrated that cox-2 plays a critical role in bone resorption (Okada et al. 2000). Moreover, it has been shown that cox-2 is required for both intramembranous and endochondral bone formation after a fracture (Zhang et al. 2002) and that cox-2 function is essential for fracture healing (Simon et al. 2002). In several experimental studies, cox-2 inhibitors have been found to affect bone metabolism and to delay fracture healing (Simon et al. 2002, Bergenstock et al. 2005, Endo et al. 2005, Gerstenfeld et al. 2007, Simon and O'Connor 2007), a potential clinical problem in fracture patients. However, the effects of parecoxib on bone metabolism have not been sufficiently investigated. There is still controversy regarding the possible negative effects of cox-2 inhibitors on fracture healing compared to those of the conventional cox inhibitors, and two published reports have claimed that this effect is less with cox-2-specific inhibitors (Gerstenfeld et al. 2003, Brown et al. 2004). Aspenberg (2004), however, suggested that the negative effects of cox-2 inhibitors on bone healing may have been underestimated because of pharmacokinetic differences in research animals.

It is of major interest to establish whether or not the selective cox-2 inhibitors can be used safely in orthopedic fracture treatment. Considering that parecoxib may well become the anti-inflammatory drug of choice for parenteral treatment of postoperative pain, this study was designed to investigate the effects of parecoxib on fracture healing. Our hypothesis was that parecoxib impairs fracture healing.

Material and methods

Experimental animals

52 adult female Wistar rats (Taconic Europe, Lille Skensved, Denmark) with a mean weight of 240 (213–277) g were randomly allocated into an experimental group and a control group. Pairs of animals, one from each group, were kept in wire-topped plastic cages with free access to tap water and standard laboratory rodent chow (with 1.1% calcium, 0.8 % phosphorus, and 1,500 IU/kg vitamin D3) in a cycle of 12 hours light and 12 hours dark. For surgery and bone density measurements, the animals were anesthetized with a combination of Hypnorm (fluanisone 5 mg/mL, fentanyl citrate 0.1575 mg/mL) and Dormicum (midazolam 2.5 mg/mL). These agents were administered subcutaneously at a dose of 0.2 mL/100 g body weight for surgery and 0.15 mL/100 g body weight for bone density measurements. Temgesic (buprenorphin 0.3 mg/mL) at 0.01 mL/100 g body weight was given subcutaneously immediately after surgery, on the first postoperative day, and later when necessary. The experiment conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes, and consent was obtained from this organization.

Tibial fracture

The surgical model has been used previously by our group (Madsen et al. 1998). Surgery was performed under sterile conditions. A stab incision was made through the patellar tendon of the right tibia of anesthetized animals. The cannula from a ven-

flon (BD venflon Pro, 1.3 × 32 mm) was inserted through the anterior tibial plateau just medial to the patellar tendon in front of the cruciate ligaments. A cannula (Braun Sterican, 1.20 × 40 mm) and the stylet of a spinal needle (Braun Spinocan, 0.70 × 88 mm) were then inserted into the largest cannula. All 3 were advanced as far distally as possible by combined axial pressure and rotation. The outer 2 cannulas were then withdrawn to the proximal part of the tibia. With the stylet remaining inside, the tibia was subjected to a standardized closed mid-shaft fracture using a specially designed fracture forceps (Ekeland et al. 1981). The 2 cannulas were then advanced distally over the stylet, past the fracture line into the distal tibial metaphysis. Rotation was checked by comparing the alignment of the foot and thigh. The nail was cut flush with the tibial plateau and the skin was closed with one suture. All fractures were assessed to be stable by manual testing at the end of the operation.

Parecoxib administration

All animals in the experimental group were given a standard dose of parecoxib at 0.05 mg/100 g body weight intraperitoneally in the morning and in the evening for 7 days, with the first injection immediately before surgery. The animals in the placebo group were given a corresponding volume of saline intraperitoneally at the same time points.

Tissue processing

1 animal from the parecoxib group had to be killed after 2 weeks because of fixation failure. 10 animals (5 from each group) were killed after anesthesia with in vivo perfusion after 3 weeks and 5 other animals from each group were killed after 6 weeks to be included in a pilot study characterizing the tissues at the cellular and molecular levels. The remaining 31 animals were killed from a pentobarbital overdose after 6 weeks. Both tibias were resected, with care being taken to leave the periosteum over the fracture, and the nails were removed from the fractured tibias before mechanical testing. One animal from the parecoxib group was excluded due to fracture of the tibia on removal of the nail. The bones were frozen wet in Ringer acetate solution at –20°C to await further processing (Madsen et al. 1998).

Bone density measurements

2 and 3 weeks after surgery, the bone mineral density (BMD) at the fracture site was measured on anesthetized animals using dual-energy X-ray absorptiometry (DEXA) with a bone densitometer specially designed for measurements on small animals (Lunar PIXImus; GE Lunar Corp., Madison, WI). 6 weeks after surgery, BMD at the fracture site of the remaining 41 animals was measured. The smallest possible quadratic area of measurement with the PIXImus software was placed anteriorly and aligned with the longitudinal axis of the tibia over the fracture including the medial cortex, the callus, and the nail. This procedure was performed twice, and the mean BMD was calculated. The nail was measured alone and its constant value was subtracted from the BMD values calculated, in order to obtain an estimate of the real BMD at the fracture site.

Mechanical testing

For mechanical testing, the tibias of the remaining 30 animals were thawed in Ringer acetate solution. Each fractured right tibia was mounted in a specially designed testing jig and loaded in an MTS machine until fracture (Minin Bionix model 858 with TestStar II controller; MTS Systems, Eden Prairie, MN) in three-point ventral cantilever bending at a rate of 7.2 degrees per second with the fulcrum placed over the fracture callus (Nordsletten et al. 1994). The ultimate bending moment, ultimate energy absorption, bending stiffness, and deflection were registered in TestStar II software. The corresponding intact left tibia was fractured at the same level, and for each animal the ratio between mechanical values for affected and non-affected bone was calculated. Nonunions were given zero values for moment, energy and stiffness, and 86.4° for deflection (maximal bending when the test was terminated after 12 seconds).

Statistics

Calculations were done using SPSS 13. Results are given as mean values, and dispersion is expressed as one standard deviation (SD) and 95% confidence intervals. The mean BMD after 2, 3, and 6 weeks for each animal was calculated and the mean BMD for all animals in the 2 groups were compared.

Table 1. Bone mineral density (g/cm^2) values of the fracture area during the 6-week period after tibial fracture and intramedullary nailing. The level of significance after Bonferroni adjustment was p < 0.02

Time from fracture	Parecoxib group mean (SD)	n	Placebo group mean (SD)	n	Mean difference	95% CI of mean difference	P-value
2 weeks	0.18 (0.06)	26	0.23 (0.04)	26	0.05	0.018–0.081	0.003
3 weeks	0.24 (0.06)	25	0.27 (0.05)	26	0.03	-0.002–0.063	0.06
6 weeks	0.30 (0.08)	20	0.33 (0.07)	21	0.03	-0.014–0.087	0.2
Mean [a]	0.23 (0.06)		0.27 (0.05)		0.04	0.010–0.071	0.01

[a] Mean for 2–6 weeks

Table 2. Ratios for mechanical properties between fractured and intact tibias at 6 weeks after fracture and intramedullary nailing

	Parecoxib (n = 14) mean (SD)	Placebo(n = 16) mean (SD)	Mean difference	95% CI of mean difference	P-value
Moment (ratio)	0.41 (0.33)	0.49 (0.30)	0.08	-0.35–0.18	0.5
Total energy (ratio)	0.40 (0.33)	0.49 (0.31)	0.09	-0.35–0.18	0.5
Stiffness (ratio)	0.58 (0.44)	0.74 (0.44)	0.16	-0.54–0.21	0.4
Deflection (ratio)	0.89 (0.50)	0.62 (0.23)	0.27	-0.31–0.58	0.08

The DEXA measurements and mechanical testing parameters were the primary endpoints of the study. The mechanical testing was performed at one time point; no multiplicity adjustments were made. To reduce the type-1 error rate we made multiplicity adjustments for the DEXA measurements, which were performed at 3 time points. The groups were compared by unpaired Student's t-test with Bonferroni adjustment for multiple tests. For the mechanical tests, the level of significance was set at $p < 0.05$; for the DEXA measurements $p < 0.05/3$ ($p < 0.02$) after Bonferroni adjustment.

Results

Bone density measurements

Mean BMD at the fracture site was lower in the parecoxib group than in the control group: 0.23 (SD 0.06) g/cm^2 and 0.27 (SD 0.05) g/cm^2, respectively ($p = 0.01$) (Table 1). At each time point, the mean BMD at the fracture site was lower in the parecoxib group than in the control group. The difference between the groups became less with time. After 2 weeks, the mean BMD at the fracture site in the parecoxib group was 0.18 (SD 0.06) g/cm^2 and in the placebo group it was 0.23 (SD 0.04) g/cm^2, a reduction of 22% in the parecoxib group. After 3 weeks, the corresponding values were 0.24 (SD 0.06) g/cm^2 and 0.27 (SD 0.05) g/cm^2, a difference of 11%. After 6 weeks, immediately before the animals were killed, mean BMD in the parecoxib group was 0.30 (SD 0.08) g/cm^2 and in the placebo group it was 0.33 (SD 0.07) g/cm^2. The difference between groups had thus decreased to 9%.

Mechanical testing

There were no statistically significant differences in mechanical properties between the parecoxib group and the placebo group after 6 weeks (Table 2). 2 nonunions occurred in the parecoxib group.

Calculation of the bending moment required for fracture of the tibias gave a mean ratio between the fractured right tibia and the left tibia of 0.41 (SD 0.33) in the parecoxib group. The corresponding ratio in the placebo group was 0.49 (SD 0.30), the reduction in the parecoxib group thus being 16%. The mean ratio for the total energy required in the parecoxib group was 0.40 (SD 0.33) and in the placebo group it was 0.49 (SD 0.31), a reduction of 18%. The mean ratio between tibias for stiffness was 0.58 (SD 0.44) in the parecoxib group and

0.74 (SD 0.44) in the placebo group, a reduction of 22%. The mean ratio between tibias for deflection was 0.89 (SD 0.50) in the parecoxib group and 0.62 (SD 0.23) in the placebo group, an increase of 44%. None of these differences reached statistical significance, however.

Discussion

Using a rat tibial fracture model, we have shown that when parecoxib was given for 1 week perioperatively in doses analogous to those used in humans, BMD at the fracture site became reduced. The effect was greatest during the first weeks of healing. When stratifying the data at each time point of follow up, i.e. comparing the 2 groups after 2, 3, and 6 weeks with t-test, we found statistically significant differences after 2 weeks, borderline differences after 3 weeks, and differences that were not significant after 6 weeks. This is consistent with the findings of Simon and O'Connor (2007), who found a statistically significant impairment in radiographic scoring after 8 weeks in animals that had received a cox-2 inhibitor for 15 days or more after fracture, but not in those that had received the inhibitor for only 5 days. We administered parecoxib for 7 days, and for this reason it is not reasonable to believe that BMD should be affected after 6 weeks. The mechanical strength was reduced after 6 weeks, but the study lacked sufficient statistical power to show the statistical significance of this finding.

The BMD results in our study correspond to the use of a tibial osteotomy model by Beck et al. (2003), where BMD in the healing area of a tibial osteotomy was found to be lower in animals given diclofenac—a conventional cox inhibitor—compared to a placebo group and to a tramadol group after 3 weeks. In that study, BMD was measured with computer tomography performed after killing and nail removal. On the other hand, we were able to measure bone density at several time points using DEXA with a bone densitometer specifically designed for measurements on small animals. To our knowledge, our study is the first to show a change in BMD over time, and with a reduction tendency. We found that the difference in BMD between the parecoxib group and the placebo group was greatest at 2 weeks. After that, the difference decreased with time—from 22% after 2 weeks to 9% after 6 weeks—which suggests that the difference in BMD between the groups would eventually disappear.

Concerning the mechanical properties, we were unable to detect statistically significant differences in mechanical strength after 6 weeks. However, we noted a trend towards reduced mechanical properties in the parecoxib group; the healing fractures in the placebo group scored higher than the parecoxib group for bending moment, energy absorption, and stiffness, and they scored lower for deflection. Furthermore, the parecoxib group contained 2 nonunions after 6 weeks whereas there were none in the placebo group. These findings may indicate that parecoxib given postoperatively for 1 week delayed fracture healing, but that our study lacked sufficient power to verify this mechanically.

Parecoxib dosage calculations are important in animal studies. As the quick celecoxib and rofecoxib metabolism seen in rats (Halpin et al. 2000, Paulson et al. 2000) may also apply to parecoxib, the dosage of parecoxib in our study may be uncertain. Meunier and Aspenberg (2006) used subcutaneous mini-pumps with continuous release of parecoxib in doses as high as 6.4 mg/kg daily to compensate for the fast metabolism. In a recent study by Gerstenfeld et al. (2007), a dose of 5.0 mg/kg daily was used whereas in a previous study they used 0.3 mg/kg daily as low dose and 1.5 mg/kg daily as high dose (Gerstenfeld et al. 2003). Different cox inhibitors have different pharmacological effects, and no direct correlation has been observed between the plasma concentration of cox inhibitors and the magnitude of the pharmacological effects in chronic inflammatory conditions (Huntjens et al. 2005). This might also be the case regarding inhibition of inflammation by these drugs after fracture, which would make the dose calculations even more uncertain. We chose to administer 1.0 mg/kg daily to our animals, which corresponds to the recommended human dosage.

We used female rats, and parecoxib was given intraperitoneally twice a day to ensure optimal absorption and sufficient blood concentration. The first dose was given immediately before operation and the medication was continued for a week. For this reason, our method resembles the use of cox-2 inhibitors in human trauma. Using this method, we

were able to confirm that parecoxib given for only a few days affects bone metabolism.

Previous studies have indicated that the effect of conventional cox inhibitors on mechanical strength becomes reduced with time after injury, as compared to controls. In one study, after 4 weeks the mechanical strength in animals given indomethacin was lower than in those given placebo, whereas there were no differences after 12 weeks (Brown et al. 2004). In accordance with this, ketorolac was found to have a negative effect on fracture healing at 4 weeks, but not after 8 weeks in mice (Mullis et al. 2006) and at 3 weeks, but not after 5 weeks in rats (Gerstenfeld et al. 2003). In their recent study, Gerstenfeld et al. (2007) also demonstrated that the effects of valdecoxib and ketorolac on impairment of fracture healing were reversible after short-term treatment. In our study, the mechanical fracture strength may have followed the same pattern as observed with BMD in the healing fractures; that is, there may have been differences in strength between the 2 groups earlier in the course of fracture healing. This matter could not, however, be investigated using our study design.

The safety of using cox inhibitors in the management of fracture patients has been discussed extensively. Two editorials have asserted that these drugs should be avoided during the period of bone healing because they may possibly lead to delayed fracture union (Aspenberg 2002, Einhorn 2002). Our findings support this view.

Contributions of authors

SD, LE, LN, and JEM were responsible for the design of the study. SD and JEM operated the animals and performed the DEXA measurements. SD, JEM, and HS did the programming of the MTS machine and the mechanical testing. SD, LN and JEM performed the statistical calculations. SD wrote the draft manuscript and all authors contributed to revision of the manuscript.

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No competing interests declared.

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