NSAID's and Tissue Repair – 2003 – 2006 References

This document includes a range of published material relating to the relationship between NSAID's and various aspects of tissue repair. It is not intended as a complete bibliography but as an aide for those researching this area of work

** **

Cohen, D. B.et al. (2006)
Indomethacin and celecoxib impair rotator cuff tendon-to-bone healing
Am J Sports Med 34(3): 362-9.

BACKGROUND: Nonsteroidal anti-inflammatory drugs are commonly prescribed after rotator cuff repair. These agents can impair bone formation, but no studies have evaluated their impact on tendon-to-bone healing. HYPOTHESIS: Traditional nonselective nonsteroidal anti-inflammatory drugs and cyclooxygenase-2-specific nonsteroidal anti-inflammatory drugs interfere with tendon-to-bone healing. STUDY DESIGN: Controlled laboratory study. METHODS: One hundred eighty Sprague-Dawley rats underwent acute rotator cuff repairs. Postoperatively, 60 rats received 14 days of celecoxib, a cyclooxygenase-2-specific nonsteroidal anti-inflammatory drug; 60 received indomethacin, a traditional nonselective nonsteroidal anti-inflammatory drug; and 60 received standard rat chow. Animals were sacrificed at 2, 4, and 8 weeks and evaluated by gross inspection, biomechanical testing, histologic analysis, and polarized light microscopy to quantify collagen formation and maturation. RESULTS: Five tendons completely failed to heal (4 celecoxib, 1 indomethacin). There were significantly lower failure loads in the celecoxib and indomethacin groups compared with the control groups at 2, 4, and 8 weeks (P <.001), with no significant difference between nonsteroidal anti-inflammatory drug groups. There were significant differences in collagen organization and maturation between the controls and both nonsteroidal anti-inflammatory drug groups at 4 and 8 weeks (P <.001). Controls demonstrated progressively increasing collagen organization during the course of the study (P <.001), whereas the nonsteroidal anti-inflammatory drug groups did not. CONCLUSION: Traditional and cyclooxygenase-2-specific nonsteroidal anti-inflammatory drugs significantly inhibited tendon-to-bone healing. This inhibition appears linked to cyclooxygenase-2. CLINICAL RELEVANCE: If the results of this study are verified in a larger animal model, the common practice of administering non-steroidal anti-inflammatory drugs after rotator cuff repair should be reconsidered.

Tsai, W.et al. (2006)
Ibuprofen inhibition of tendon cell migration and down-regulation of paxillin expression.
J Orthop Res 24(3): 551-8.

Sports-related tendon injuries are commonly treated with nonsteroidal antiinflammatory drugs. Tendon healing requires migration of tendon cells to the repair site, followed by proliferation and synthesis of the extracellular matrix. This study was designed to determine the effect of ibuprofen on the migration of tendon cells intrinsic to rat Achilles tendon. Whether a correlation exits between this effect and the expression of paxillin, which is a positive regulator of cell spreading and migration, was also investigated. The migration of tendon cells was evaluated ex vivo by counting the number of initial outgrowths from the tendon explants and in vitro by transwell filter migration assay. The spreading of tendon cells in culture was also evaluated microscopically. The mRNA and protein expressions of paxillin were determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. Dose-

dependent ibuprofen inhibition was demonstrated on the migration of tendon cells both ex vivo, and in vitro. Similar inhibition was also observed on the spreading of tendon cells. Suppression of mRNA expression and protein level of paxillin was revealed by RT-PCR and Western blot analyses. The expression of focal adhesion kinase (FAK) and tyrosine phosphorylation of FAK remained unchanged. In conclusion, ibuprofen inhibits tendon cell migration in a process that is probably mediated by the down-regulation of paxillin.

Salcido, R. S. (2005)
Do anti-inflammatories have a role in wound healing?
Adv Skin Wound Care 18(2): 65-6.

Endo, K. et al. (2005)
Cyclooxygenase-2 inhibitor delays fracture healing in rats
Acta Orthop 76(4): 470-4.

BACKGROUND: Cyclooxigenase-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.

Clarke, S. and F. Lecky (2005)
Best evidence topic report. Do non-steroidal anti-inflammatory drugs cause a delay in fracture healing?
Emerg Med J 22(9): 652-3.

A short cut review was carried out to establish whether there is any evidence that non-steroidal anti-inflammatory drugs (NSAIDs) might delay fracture healing. A total of 514 papers were found using the reported search, of which three represent the best evidence to answer the clinical question. The author, date and country of publication, patient group studied, study type, relevant outcomes, results, and study weaknesses of these best papers are tabulated. At present, although there are theoretical concerns about the adverse effects of NSAIDs on fracture healing, there is not enough clinical evidence to deny patients with simple fractures their analgesic benefits.

Radi, Z. A. and N. K. Khan (2005)
Effects of cyclooxygenase inhibition on bone, tendon, and ligament healing
Inflamm Res 54(9): 358-66.

Cyclooxygenases (COX-1 and COX-2) catalyze the conversion of arachidonic acid to prostaglandins (PGs). PGs play a significant role in bone metabolism in health and disease. Conventional non-selective, non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (COXIBs) are widely used in patients with musculoskeletal conditions and as a post-surgical analgesics. Due to their effects on PG synthesis, these drugs have been hypothesized to affect the healing process of bone fractures and orthopedic surgical sites. A

variety of experimental models of bone, ligament, and tendon repair have assessed the effects of these selective and non-selective COX inhibitors in animals, but with variable outcomes. At this time, large-scale, robust clinical study data do not exist, limiting the relevant assessment of experimental animal data to humans. Here, we provide a critical review of available data on the role of PGs and the effect of COX inhibitors on bone, tendon, and ligament repair. Collectively, this assessment suggests potential involvement of PGs in the healing process of these tissues via modulation by non-selective NSAIDs and selective COX-2 inhibitors.

Beck, A.et al. (2005)
Nonsteroidal anti-inflammatory drugs (NSAIDs) in the perioperative phase in traumatology and orthopedics effects on bone healing
Oper Orthop Traumatol 17(6): 569-78.

OBJECTIVE: To achieve analgesic, anti-inflammatory and antipyretic effects in traumatology and orthopedic surgery without side effects or with the least possible side effects, with special emphasis on bone healing. INDICATIONS: Acute and chronic inflammatory conditions, e. g., rheumatoid arthritis, ankylosing spondylitis. Degenerative joint disease. Posttraumatic and postoperative pain, edema, or fever. Prevention of heterotopic bone formation. CONTRAINDICATIONS: Hypersensitivity. Gastrointestinal ulceration or bleeding. Severe hepatic or renal impairment. RESULTS: Nonsteroidal anti-inflammatory drugs (NSAIDs) are invaluable in treating a variety of musculoskeletal conditions. As well as their excellent analgesic potency their anti-inflammatory effects are beneficial in treating posttraumatic and postoperative edema. In addition, NSAIDs inhibit heterotopic bone formation after hip arthroplasty. Animal studies, however, have demonstrated that they cause delayed fracture healing. Although clinical studies have not yet supplied unequivocal evidence of this effect in human subjects, the authors recommend that in the presence of other risk factors which may adversely affect fracture healing, such as smoking, diabetes mellitus or peripheral arterial occlusive disease, the indication of NSAID use for analgesia should be strictly limited. Therapeutic alternatives such as centrally acting agents (e. g., weak opioids) should be considered in these patients.

Bhattacharyya, T.et al. (2005)
Nonsteroidal antiinflammatory drugs and nonunion of humeral shaft fractures
Arthritis Rheum 53(3): 364-7.

OBJECTIVE: To analyze the relationship between nonunion of humeral shaft fractures and nonsteroidal antiinflammatory drug (NSAID) exposure in older adults. METHODS: A cohort of 9,995 patients with humeral shaft fractures was identified using diagnosis and procedure codes from a Medicare database of >500,000 patients. Prescription NSAID as well as prescription opioid use was assessed from pharmacy claims data for 3 30-day periods immediately after the initial fracture. Nonunion was defined by the presence of procedure codes for repair of nonunion 90-365 days after the index fracture. We examined the association between NSAIDs and nonunion using multivariate Cox proportional hazards models. RESULTS: Of the 9,995 humeral shaft fractures, 105 patients developed nonunions (1.1%), and 1,032 (10.3%) were exposed to NSAIDs in the 90 days after fracture. NSAID exposure within the first 90 days was significantly associated with nonunion (relative risk [RR] 3.7, 95% confidence interval [95% CI] 2.4-5.6). When indicators for exposure to NSAIDs during each of the 3 30-day windows were placed into the same multivariate model, only the period 61-90 days post-fracture was significantly associated with nonunion (RR 3.9, 95% CI 2.0-6.2). We observed a similar association between opioids and nonunion, with exposure to opioids between 61 and 90 days associated with nonunion (RR 2.7, 95% CI 1.5-5.2), but exposure to opioids during neither of the 2 earlier 30-day periods significantly associated with nonunion. CONCLUSION: We found that exposure to nonselective NSAIDs or opioids in the

period 61-90 days after a humeral shaft fracture was associated with nonunion. Although these associations may be causal, they are more likely to reflect the use of analgesics by patients with painful nonhealing fractures.

Hanson, C. A.et al. (2005)
The effect of analgesic agents on the healing rat medial collateral ligament
Am J Sports Med 33(5): 674-9.

BACKGROUND: Studies have suggested that some nonselective nonsteroidal anti-inflammatory drugs, including piroxicam, may improve ligament healing, whereas other nonsteroidal anti-inflammatory drugs, including ibuprofen and the cyclooxygenase-2 inhibitor celecoxib, may have no effect on the mechanical properties or may even deter the healing process. These results might reflect variations in cyclooxygenase enzyme selectivity by different drugs or, alternatively, may be related to their analgesic properties because it is generally accepted that early activity improves ligament healing. HYPOTHESIS: Nonselective nonsteroidal anti-inflammatory drugs improve ligament healing, whereas other analgesics provide lesser degrees of improvement, and cyclooxygenase-2 inhibitors are detrimental. STUDY DESIGN: Controlled laboratory study. METHODS: One hundred fifty-five Sprague-Dawley rats were divided into 7 treatment groups (piroxicam, naproxen, rofecoxib, butorphanol, 2 doses of acetaminophen, and control). The right medial collateral ligament of each rat was transected, and the drugs were administered postoperatively on days 1 to 6. On day 14, the rats were sacrificed, and mechanical testing was performed on the medial collateral ligament. RESULTS: The piroxicam group demonstrated significantly greater load to failure (27%) compared with the control. No significant differences were observed between other groups. CONCLUSIONS: Piroxicam improves ligament healing, but this effect cannot be attributed to all nonselective nonsteroidal anti-inflammatory drugs. Opiate analgesics, acetaminophen, and cyclooxygenase-2 inhibitors do not appear to categorically affect ligament healing. CLINICAL RELEVANCE: In the treatment of ligament injury, piroxicam may be a drug of choice.

Wheeler, P. and M. E. Batt (2005).
Do non-steroidal anti-inflammatory drugs adversely affect stress fracture healing? A short review.
Br J Sports Med 39(2): 65-9.

A literature search was performed to determine whether non-steroidal anti-inflammatory drugs (NSAIDs) adversely affect the healing of stress fractures. Evidence exists from laboratory studies and animal subjects that NSAIDs can affect fracture healing. This link has not been proved or disproved in human subjects, particularly for stress fractures. In view of the high usage of NSAIDs in treating musculoskeletal disorders, research is required to investigate whether the healing of stress fractures is affected by these drugs.

Brown, K. M.et al. (2004)
Effect of COX-2-specific inhibition on fracture-healing in the rat femur
J Bone Joint Surg Am 86-A(1): 116-23.

BACKGROUND: Nonsteroidal anti-inflammatory medications have been shown to delay fracture-healing. COX-2-specific inhibitors such as celecoxib have recently been approved for human use. Our goal was to determine, mechanically, histologically, morphologically, and radiographically, whether COX-2-specific inhibition affects bone-healing. METHODS: A nondisplaced unilateral fracture was created in the right femur of fifty-seven adult male rats. Rats were given no drug, indomethacin (1 mg/kg/day), or celecoxib (3 mg/kg/day) daily, starting on postoperative day 1. Fractures were analyzed at four, eight, and twelve weeks after creation of the fracture. Callus and bridging bone formation was assessed

radiographically. The amounts of fibrous tissue, cartilage, woven bone, and mature bone formation were determined histologically. Morphological changes were assessed to determine fibrous healing, callus formation, and bone-remodeling. Callus strength and stiffness were assessed biomechanically with three-point bending tests. RESULTS: At four weeks, only the indomethacin group showed biomechanical and radiographic evidence of delayed healing. Although femora from rats treated with celecoxib appeared to have more fibrous tissue than those from untreated rats at four and eight weeks, radiographic signs of callus formation, mechanical strength, and stiffness did not differ significantly between the groups. By twelve weeks, there were no significant differences among the three groups. CONCLUSIONS: Postoperative administration of celecoxib, a COX-2-specific inhibitor, did not delay healing as seen at twelve weeks following fracture in adult rat femora. At four and eight weeks, fibrous healing predominated in the celecoxib group as compared with the findings in the untreated group; however, mechanical strength and radiographic signs of healing were not significantly inhibited. Clinical Relevance: Many orthopaedists rely on narcotic analgesia for postfracture and postoperative pain, despite deleterious side effects and morbidity. Traditional nonsteroidal anti-inflammatory medications have been shown to delay fracture union. This effect may be smaller with COX-2-specific inhibitors.

Gerstenfeld, L. C. and T. A. Einhorn (2004).
COX inhibitors and their effects on bone healing.
Expert Opin Drug Saf 3(2): 131-6.

Prostaglandins (PGs) are released as part of the inflammatory response. They are synthesised from arachidonic acid by the cyclooxygenase enzymes, COX-1 and -2. NSAIDS inhibit COX activity and have become the primary means of alleviating chronic pain associated with rheumatoid and osteoarthritis. They are also widely used as analgesics in the treatment of acute postsurgical and traumatic pain. PGs are known to play important functions in bone repair and normal bone homeostasis. Animal studies suggest that, whilst both nonspecific and specific inhibitors of COXs impair fracture healing, some studies have suggested that this impairment is due to COX-2. Although these data raise concerns about the use of COX-2-specific inhibitors as anti-inflammatory or analgesic drugs in patients undergoing orthopaedic procedures, clinical reports have been largely inconclusive concerning the effects of NSAIDs on bone healing. Since animal data suggest that the effects of COX-2 inhibitors are both dose-dependent and reversible, in the absence of scientifically sound clinical evidence it is suggested that physicians consider short-term administration or use of other drugs in the management of these patients.

Aspenberg, P. (2004)
Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs
J Orthop Res 22(3): 684; author reply 685.

Seidenberg, A. B. and Y. H. An (2004)
Is there an inhibitory effect of COX-2 inhibitors on bone healing?
Pharmacol Res 50(2): 151-6.

The use of the new selective cyclooxygenase-2 (COX-2) inhibitors (such as celecoxib and rofecoxib) for the treatment of pain and inflammation caused by fractures, cementless total joint replacements, soft tissue healing to bone, and spinal fusion surgeries has been controversial due to the convincing data collected from nonspecific NSAIDs such as indomethacin and naproxen regarding their inhibitory effects on bone healing and the similar effects of COX-2 specific NSAIDs in animal models. Is there a significant inhibitory effect of

COX-2 inhibitors on bone healing in humans? To answer this question, we reviewed existing scientific evidence (based mainly on a MedLine search) of the potential effects of COX-2 inhibitors on bone healing. The literature shows that COX-2 inhibitors do have inhibitory effects on bone healing in animal models, but the effects of COX-2 inhibitors on similar processes in humans remain largely unknown.

Mason, L., R. A. Moore, et al. (2004)
Topical NSAIDs for acute pain: a meta-analysis.
BMC Fam Pract 5: 10.

BACKGROUND: A previous systematic review reported that topical NSAIDs were effective in relieving pain in acute conditions like sprains and strains, with differences between individual drugs for efficacy. More trials, a better understanding of trial quality and bias, and a reclassification of certain drugs necessitate a new review. METHODS: Studies were identified by searching electronic databases and writing to manufacturers. We selected randomised double blind trials comparing topical NSAID with either placebo or another active treatment in adults with acute pain, and extracted dichotomous information approximating to a 50% reduction in pain at one week, together with details of adverse events and withdrawals. Relative benefit and number-needed-to-treat (NNT), and relative risk and number-needed-to-harm (NNH) were calculated, with sensitivity analyses where appropriate to investigate differences between individual drugs and aspects of trial design. RESULTS: Twenty-six double blind placebo controlled trials had information from 2,853 patients for evaluation of efficacy. Topical NSAID was significantly better than placebo in 19 of the 26 trials, with a pooled relative benefit of 1.6 (95% confidence interval 1.4 to 1.7), and NNT of 3.8 (95% confidence interval 3.4 to 4.4) compared with placebo for the outcome of half pain relief at seven days. Results were not affected by outcome reported, or condition treated, but smaller trials yielded a larger estimate of efficacy. Indirect comparisons of individual topical NSAIDs showed that ketoprofen was significantly better than all other topical NSAIDs, while indomethacin was barely distinguished from placebo. Three trials, with 433 patients, compared topical with oral NSAID (two trials compared the same drug, one compared different drugs) and found no difference in efficacy. Local adverse events, systemic adverse events, or withdrawals due to an adverse event were rare, and no different between topical NSAID and placebo. CONCLUSIONS: Topical NSAIDs were effective and safe in treating acute painful conditions for one week.

Mason, L., R. A. Moore, et al. (2004)
Topical NSAIDs for chronic musculoskeletal pain: systematic review and meta-analysis.
BMC Musculoskelet Disord 5: 28.

A previous systematic review reported that topical NSAIDs were effective in relieving pain in chronic conditions like osteoarthritis and tendinitis. More trials, a better understanding of trial quality and bias, and a reclassification of certain drugs necessitate a new review. METHODS: Studies were identified by searching electronic databases, and writing to manufacturers. We identified randomised, double blind trials comparing topical NSAID with either placebo or another active treatment, in adults with chronic pain. The primary outcome was a reduction in pain of approximately 50% at two weeks, and secondary outcomes were local and systemic adverse events and adverse event-related withdrawals. Relative benefit and number-needed-to-treat (NNT), and relative harm and number-needed-to-harm (NNH) were calculated, and the effects of trial quality, validity and size, outcome reported, and condition treated, were examined by sensitivity analyses. RESULTS: Twelve new trials were added to 13 trials from a previous review. Fourteen double blind placebo-controlled trials had information from almost 1,500 patients. Topical NSAID was significantly better than placebo with relative benefit 1.9 (95% confidence interval 1.7 to 2.2), NNT 4.6 (95% confidence interval 3.8 to 5.9). Results were not affected by trial quality, validity or size, outcome reported, or condition treated. Three

trials with 764 patients comparing a topical with an oral NSAID found no difference in efficacy. Local adverse events (6%), systemic adverse events (3%), or the numbers withdrawing due to an adverse event were the same for topical NSAID and placebo. CONCLUSIONS: Topical NSAIDs were effective and safe in treating chronic musculoskeletal conditions for two weeks. Larger and longer trials are necessary to fully elucidate the place of topical NSAIDs in clinical practice.

Virchenko, O.et al. (2004)
Parecoxib impairs early tendon repair but improves later remodeling
Am J Sports Med 32(7): 1743-7.

BACKGROUND: Cyclooxygenase-2 inhibitors inhibit bone repair. HYPOTHESIS: Cyclooxygenase inhibitors might also have a negative effect on early tendon repair, although a positive effect on late tendon repair previously has been shown. STUDY DESIGN: Controlled laboratory study. METHODS: Achilles tendon transection was performed on 80 rats. Sixty rats were given daily intramuscular injections of either parecoxib (6.4 mg/kg body weight) or saline for the first 5 days after surgery and sacrificed either at 8 or 14 days. The remaining 20 rats were given intramuscular parecoxib or saline injections from day 6 until sacrifice at 14 days. RESULTS: At 8 days, early parecoxib treatment caused a 27% decrease in force at failure (P =.007), a 25% decrease in maximum stress (P =.01), and a 31% decrease in energy uptake (P =.05). Stiffness and transverse area were not significantly affected. At 14 days, early parecoxib treatment caused a decrease in stiffness (P =.004). In contrast to early treatment, late parecoxib treatment caused a 16% decrease in cross-sectional area (P =.03) and a 29% increase in maximum stress (P =.04). CONCLUSIONS: During early tendon repair, a cyclooxygenase-2 inhibitor had a detrimental effect. During remodelling, however, inflammation appears to have a negative influence, and cyclooxygenase-2 inhibitors might be of value. CLINICAL RELEVANCE: The results suggest that cyclooxygenase-2 inhibitors should be used with care in the early period after tendon injury.

Rahusen, F. T.et al. (2004)
Nonsteroidal anti-inflammatory drugs and acetaminophen in the treatment of an acute muscle injury
Am J Sports Med 32(8): 1856-9.

BACKGROUND: Nonsteroidal anti-inflammatory drugs are frequently used to treat muscle injuries in athletes. It is not known whether the anti-inflammatory effects of these drugs are important or whether their effectiveness is a result of their central analgesic effect. HYPOTHESIS: The effects of nonsteroidal anti-inflammatory drugs are no different than the effects of an analgesic (acetaminophen) without anti-inflammatory action in an experimental, acute muscle contusion model. STUDY DESIGN: Controlled animal study. METHODS: A standardized, unilateral, nonpenetrating injury was created to the tibialis anterior muscle of 96 adult male mice. Four treatment groups were used: group 1, placebo treatment; group 2, treatment with rofecoxib, a nonsteroidal anti-inflammatory drug with cyclooxygenase-2 selectivity, and treatment after the injury; group 3, rofecoxib treatment starting 24 hours before the injury; and group 4, acetaminophen treatment after the injury. The muscle and the contralateral normal muscle were evaluated at 2, 5, and 7 days after injury by grading of gait, wet weight as a measure of edema, and histologic evaluation. RESULTS: Group 1 had significantly more gait disturbances at day 2 than all other groups (P <.05). No differences were found at days 5 and 7. Wet weights showed an increase at day 2 in group 1 (P <.01). Again, no differences were found at days 5 and 7. Histology revealed similar inflammatory changes at day 2 in all groups, with regeneration of muscle fibers at days 5 and 7. CONCLUSIONS: The results indicate that rofecoxib as a nonsteroidal anti-inflammatory drug and acetaminophen as a non-nonsteroidal anti-inflammatory drug analgesic have similar effects. The lack of differences in wet weights and histology suggests that the anti-

inflammatory effects of rofecoxib are not an important feature of its action. CLINICAL RELEVANCE: The routine use of nonsteroidal anti-inflammatory drugs in muscle injuries may need to be critically evaluated because low-cost and low-risk analgesics may be just as effective.

Tsai, W. C.et al. (2004)
Ibuprofen inhibition of tendon cell proliferation and upregulation of the cyclin kinase inhibitor p21CIP1
J Orthop Res 22(3): 586-91.

Sports-related tendon injuries are commonly treated with nonsteroidal antiinflammatory drugs. This study was designed to determine the in vitro effect of ibuprofen on the proliferation of tendon cells intrinsic to rat Achilles tendon. Furthermore, the existence of a correlation between this effect and the expression of the cyclin kinase inhibitor p21(CIP1) and retinoblastoma (Rb) protein was also examined. Using cultured tendon cells, cell viability was evaluated by MTT assay. To determine whether apoptosis was related to the effect of ibuprofen, terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) assay was used. The mitotic index (MI) was calculated from the number of cells in the mitotic phase as stained and identified by propidium iodide. The mRNA expression of p21(CIP1) was determined by reverse transcription-polymerase chain reaction (RT-PCR). Protein expressions of p21(CIP1) and Rb protein were determined by Western blot analysis. A dose-dependent decrease in the cellularity of tendon cells by ibuprofen was demonstrated by MTT assay ($p<0.001$). However, TUNEL assay revealed no evidence of apoptosis. Ibuprofen dose-dependently reduced the MI ($p<0.001$). Upregulation of p21(CIP1) both at the levels of mRNA expression and protein was revealed from RT-PCR and Western blot analyses. The inhibition of Rb protein phosphorylation was also noted in ibuprofen-treated cells. In conclusion, ibuprofen inhibits tendon cell proliferation in a process that is probably mediated by the upregulation of p21(CIP1) and reduced phosphorylation of Rb protein.

Evans, C. E. and C. Butcher (2004)
The influence on human osteoblasts in vitro of non-steroidal anti-inflammatory drugs which act on different cyclooxygenase enzymes
J Bone Joint Surg Br 86(3): 444-9.

There is increasing evidence that non-steroidal anti-inflammatory drugs (NSAIDs) can adversely affect bone repair. We have, therefore, studied the in vitro effects of NSAIDs, which differentially inhibit cyclooxygenases (COX), the prostaglandin/thromboxane synthesising enzymes, on human osteoblasts. Indomethacin and the new nitric oxide (NO)-donating NSAIDs block the activity of both COX-1 and COX-2. Indomethacin and 5,5-dimethyl-3-(3 fluorophenyl)-4-(4 methylsulphonal) phenyl-2 (5H)-furanone (DFU) reduced osteoblast numbers in a dose-dependant manner and increased collagen synthesis and alkaline phosphatase activity. The reduction in osteoblast numbers was not caused by loss of adhesion and was reversible. Neither NSAID influenced DNA synthesis. There was no difference between the effects of indomethacin and DFU. NO-NSAIDs did not affect cell numbers. These results suggest that care should be taken when administering NSAIDs to patients with existing skeletal problems and that NO-NSAIDs may be safer.

Hochberg, M. C.et al. (2003)
Cox-2 inhibitors and fracture healing: an argument against such an effect
J Bone Miner Res 18(3): 583; author reply 584-7.

Riew, K. D.et al. (2003)

Time-dependent inhibitory effects of indomethacin on spinal fusion
J Bone Joint Surg Am 85-A(4): 632-4.

BACKGROUND: The use of nonsteroidal anti-inflammatory drugs following spine arthrodesis is discouraged because of the negative effects on bone-healing. We are not aware of any data regarding when nonsteroidal anti-inflammatory drugs may be safely resumed postoperatively. We hypothesized that these drugs have a time-dependent deleterious effect on fusion, with the greatest inhibition during the early phases of fusion. METHODS: Seventy New Zealand White rabbits underwent posterior intertransverse process arthrodesis at L5-L6 with use of iliac autograft. Rabbits randomly received indomethacin (10 mg/kg orally) starting at two weeks after surgery (twenty-four animals), indomethacin starting at four weeks postoperatively (twenty-three), or saline starting at two weeks postoperatively (twenty-three) (the control group). The animals were killed at six weeks, and the spines were denuded of soft tissues and palpated for L5-L6 motion. Fusion was defined as the complete absence of motion. RESULTS: Sixty-five percent (fifteen) of the twenty-three spines in the control group and 48% (eleven) of the twenty-three in the four-week group fused. However, only 21% (five) of the twenty-four spines in the two-week group fused. The difference between the two-week and control groups was significant ($p < 0.002$), as was the difference between the two and four-week groups ($p = 0.05$). The difference between the four-week and control groups was not significant ($p = 0.2$). CONCLUSIONS: The earlier that indomethacin was resumed postoperatively, the greater was its negative effect on fusion. Indomethacin appears to play a significant inhibitory role in the early phase of healing. Initiating indomethacin treatment in the latter phase of healing does not appear to significantly affect fusion rates, although there was a nonsignificant trend toward inhibition. To our knowledge, this is the first investigation of the time-dependent nature of indomethacin's effect on bone-healing.

Kjaersgaard-Andersen, P. and K. Jensen (2003)
Cox inhibitors and bone healing
Acta Orthop Scand 74(2): 230-1; author reply 231.

Beck, A.et al. (2003)
Influence of diclofenac (group of nonsteroidal anti-inflammatory drugs) on fracture healing
Arch Orthop Trauma Surg 123(7): 327-32.

INTRODUCTION: Nonsteroidal antirheumatics (NSAR; NSAID) are often used in patients with fractured bones for analgetic reasons. This animal experiment was performed to determine the influence of NSAR on the process of fracture healing. As an alternative, tramadol, the centrally acting analgetic without peripheral effects, was included in this experiment. MATERIALS AND METHODS: Wistar rats were operated on by a transverse osteotomy of the proximal tibia of the left leg. The fracture was stabilized by intramedullary nailing (healing period 21 days). All drugs were applied orally twice a day. The animals were divided into four groups with 10 rats each: Group 1 was treated with placebo (P), group 2 with tramadol (T; 20 mg/kg body weight/day), group 3 with diclofenac sodium (DS; 5 mg/kg bw/day) for 7 days followed by 14 days of placebo, group 4 with diclofenac sodium (DL; 5 mg/kgbw/day) over 21 days. On day 21 the rats were killed, and each leg was examined by X-ray, then the tibia was examined by CT scan, three-point bending, and histology. RESULTS: The results of CT and three-point bending showed that rats treated by diclofenac presented with delayed fracture healing compared with those treated by placebo or tramadol. Bone density in CT was highest in group 1 (mean 611.4+/-50.1 mg/ml), followed by group 2 (mean 542.5+/-29.5 mg/ml). Groups 3 (mean 411+/-34.0 mg/ml; p=0.006) and 4 (mean 395.2+/-15.4 mg/ml; p=0.009) were significantly lower. The stability of the bones, as measured by the breaking force (F(max)), was highest in group 1 (mean 45.8+/-19.0 N), followed by group 2 (mean 39.0+/-7.9 N; NS); group 3 (mean 20.6+/-7.8 N; p=0.01) was significantly lower than the placebo animals,

followed by group 4 (mean 26.5+/-8.3 N; p=0.03). Similar results were shown for bending stiffness: group 1 (mean 1404.6+/-611.4 Nmm/mm), group 2 (mean 1033.2+/-232.1 Nmm/mm; NS), group 3 (mean 564.2+/-457 Nmm/mm; p=0.045), and group 4 (mean 494.8+/-340.2 Nmm/mm; p=0.028). There were no significant differences between groups 1 and 2 and between groups 3 and 4, respectively. Diclofenac serum levels on day 21 in rats with long-term diclofenac application (mean 301.4+/-83.3 ng/ml) were comparable to those in humans. CONCLUSION: Oral application of diclofenac significantly delayed fracture healing in rats. This effect might be comparable to other NSAR and fracture healing in humans.

Marsolais, D.et al. (2003)
Nonsteroidal anti-inflammatory drug reduces neutrophil and macrophage accumulation but does not improve tendon regeneration
Lab Invest 83(7): 991-9.

Whether nonsteroidal anti-inflammatory drugs have a beneficial effect on tendon regeneration is still a matter of debate. Given that inflammatory cells are thought to induce nonspecific damage following an injury, we tested the hypothesis that a 3-day treatment with diclofenac would protect tendons from inflammatory cell injury and would promote healing. Neutrophil and ED1(+) macrophage concentrations were determined in the paratenon and the core of the rat Achilles tendon following collagenase-induced injury. Hydroxyproline content, edema, and mechanical properties were also evaluated at 1, 3, 7, 14, and 28 days post-trauma. Collagenase injections induced a 70% decrease in the ultimate rupture point at Day 3. Diclofenac treatments (1 mg/kg bid) selectively decreased the accumulation of neutrophils and ED1(+) macrophages by 59% and 35%, respectively, in the paratenon, where blood vessels are numerous, but did not reduce the accumulation of neutrophils and ED1(+) macrophages in the core of the tendon. Edema was significantly reduced on Day 3 but persisted during the remodeling phase in the diclofenac-treated group only. The inhibition of leukocyte accumulation by diclofenac did not translate into a reduction of tissue damage or a promotion of tissue healing, because the mechanical properties of injured Achilles tendons were identical in placebo and diclofenac-treated groups. These results indicate that diclofenac reduced both edema and the accumulation of inflammatory cells within the paratenon but provided no biochemical or functional benefits for the Achilles tendon.

Harder, A. T. and Y. H. An (2003)
The mechanisms of the inhibitory effects of nonsteroidal anti-inflammatory drugs on bone healing: a concise review
J Clin Pharmacol 43(8): 807-15.

Nonsteroidal anti-inflammatory drug (NSAID) use continues to expand at a remarkable rate due both to the broad spectrum of clinical applications for these medications and to the relatively recent introduction of the popular COX-2-selective inhibitors. The use of NSAIDs is particularly prevalent in patients with a variety of musculoskeletal conditions and injuries. Reports of impaired bone healing associated with NSAID use, therefore, are a particular cause for concern. Animal and in vitro studies have demonstrated impaired bone healing in the presence of traditional NSAIDs, as measured by a variety of different parameters. More recently, initial studies investigating the effects of COX-2-selective inhibitors on bone healing have yielded similar results. With mounting evidence that NSAIDs do in fact interfere with proper bone healing in various animal models, questions have arisen regarding the potential mechanism through which NSAIDs produce this outcome and whether these results can be translated to clinical settings. A likely pathway for these observed effects results from an understanding of the steps involved in bone healing itself. These steps include an inflammatory response, bone resorption, and new bone formation. Investigations over the past several decades have elucidated a role for prostaglandins (PGs) in each of these areas. Specifically, PGs have been shown to elicit and participate in inflammatory responses,

increase osteoclast activity and subsequent bone resorption, and increase osteoblast activity and new bone formation. This apparent integral role for PGs in the process of bone healing, coupled with the knowledge that NSAIDs act by inhibiting the production of PGs, results in an understanding of the likely mechanism through which NSAIDs impart their deleterious effects on bone healing. By inhibiting the COX enzymes and the subsequent production of PGs, NSAIDs not only achieve their desired anti-inflammatory effects but also inhibit the increased production of PGs that is necessary for bone healing to occur. Despite this understanding of the potential mechanism through which NSAIDs inhibit bone healing in a laboratory setting, few studies exist that show whether these inhibitory effects are also evident clinically. Thus, further studies will need to decipher whether similar inhibitory effects occur in a clinical setting.

Gajraj, N. M. (2003)
The effect of cyclooxygenase-2 inhibitors on bone healing
Reg Anesth Pain Med 28(5): 456-65.

Allami, M. K. and P. V. Giannoudis (2003)
Cox inhibitors and bone healing
Acta Orthop Scand 74(6): 771-2.

Martel-Pelletier, J.et al. (2003)
Cyclooxygenase-2 and prostaglandins in articular tissues
Seminars in Arthritis and Rheumatism 33(3): 155-167.

Ankarath, S.et al. (2003)
Non steroidal anti-inflammatory drugs in orthopaedic practice: an update
Current Orthopaedics 17: 144-149.