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Naproxen Treatment Inhibits Articular Cartilage Loss in a Rat Model of Osteoarthritis

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Abstract

The effects of naproxen, a non-steroidal anti-inflammatory drug (NSAID), on articular cartilage degeneration in female Sprague Dawley rats was examined. OA was induced by destabilization of the medial meniscus (DMM) in each knee. Rats were treated with acetaminophen (60mg/kg), naproxen (8mg/kg), or 1% carboxymethylcellulose (placebo) by oral gavage twice daily for three weeks, beginning 2 weeks after surgery. OA severity was assessed by histological OARSI scoring and by measuring proximal tibia cartilage depth using contrast enhanced μCT (n=6 per group) in specimens collected at 2, 5, and 7 weeks after surgery as well as on pristine knees. Medial cartilage OARSI scores from the DMM knees of naproxen-treated rats were statistically lower (i.e., better) than the medial cartilage OARSI scores from the DMM knees of placebo-treated rats at 5-weeks (8.7 ± 3.6 vs. 13.2 ± 2.4, p=0.025) and 7-weeks (9.5 ± 1.2 vs. 12.5 ± 2.5, p=0.024) after surgery. At 5 weeks after DMM surgery, medial articular cartilage depth in the proximal tibia specimens was significantly greater in the naproxen (1.78 ± 0.26 mm, p=0.005) and acetaminophen (1.94 ± 0.12 mm, p<0.001) treated rats as compared to placebo-treated rats (1.34 ± 0.24 mm). However, at 7 weeks (two weeks after drug withdrawal), medial articular cartilage depth for acetaminophen-treated rats (1.36 ± 0.29 mm) was significantly reduced compared to specimens from the naproxen-treated rats (1.88 ± 0.14mm; p=0.004). The results indicate that naproxen treatment reduced articular cartilage degradation in the rat DMM model during and after naproxen treatment.

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Author Contributions:

David Paglia performed experiments, data analysis, and assisted with manuscript preparation. Deboleena Kanjilal optimized cartilage μCT imaging and collected and analyzed μCT image data. Yazan Kadkoy contributed to μCT analysis. Spiro Moskonas contributed to the histological and μCT analyses. Charlene Wetterstrand and Anthony Lin assisted with animal procedures. Joseph Galloway and Jeffrey Tompson performed the destabilized medial meniscus surgeries. Maya Deza Culbertson assisted with animal procedures and manuscript preparation. J. Patrick O'Connor designed the study, aided with method development, performed data analysis, and prepared the manuscript.

Keywords

Osteoarthritis; knee; rat; DMM; acetaminophen; naproxen; NSAIDs

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a large class of compounds commonly used to manage inflammation, swelling, and pain associated with osteoarthritis [1]. NSAIDs inhibit cyclooxygenase activity to prevent synthesis of pro-inflammatory prostaglandins and thereby reduce inflammation and pain [2; 3]. Two distinct cyclooxygenase enzymes, COX-1 and COX-2, are involved in prostaglandin synthesis [4-6]. Each of the many NSAIDs has different pharmacological properties and inhibits COX-1 or COX-2 to different levels [7; 8].

Whether NSAID therapy provides a direct or indirect protective effect on articular cartilage preservation in a joint compromised by acute or chronic injury remains unclear. In cultured rabbit articular chondrocytes stimulated with interleukin-1, exogenous prostaglandin E_2 reduced matrix metalloproteinase-9 (MMP-9) synthesis and conversely NSAIDs treatment (diclofenac or indomethacin) increased MMP-9 synthesis, suggesting that NSAID therapy can enable articular cartilage destruction [9]. In another in vitro study using articular chondrocytes harvested from pigs, prednisone (a steroid) was compared to piroxicam (a non-selective COX inhibitor), and to celecoxib (a selective COX-2 inhibitor) [10]. Both prednisone and celecoxib treatment decreased matrix metalloproteinase-1 (MMP-1) expression while increasing aggrecan expression. Only celecoxib treatment increased type II collagen expression. In contrast, piroxicam treatment did not affect expression of MMP-1, aggrecan, or type II collagen.

The effects of naproxen on cartilage are poorly understood, particularly in the context of injury. Naproxen inhibits COX-1 and COX-2 with near equal efficacy [7]. In an in vitro study, human articular cartilage proteoglycan content increased following 7 days of naproxen treatment [11]. Similarly, long-term administration of naproxen in adult beagles reduced neutral metalloprotease, gelatinase, and collagenase activity in articular cartilage extracts consistent with reduced proteoglycan release [12]. Naproxen treatment also prevented cartilage loss and bone erosion in a rat model of collagen-induced arthritis [13].

We hypothesize that naproxen treatment will prevent articular cartilage loss associated with osteoarthritis (OA) progression. To test the hypothesis, rats were treated with naproxen after acute destabilization of the knee medial meniscus (DMM) to induce OA. Acetaminophen, which is recommended for managing arthritis-associated pain, was used as a treatment control in the DMM-OA model [14]. In support of the hypothesis, naproxen and acetaminophen treatment prevented articular cartilage loss following the DMM procedure. However, naproxen treatment but not acetaminophen treatment had a persistent effect on preventing articular cartilage loss for two weeks following cessation of drug treatment.

Methods

Animal Model

Forty-eight female Sprague-Dawley rats that were 105-108 days old at the time of surgery were used. All procedures complied with animal welfare guidelines and were approved by the local animal care and use committee (protocol #201800021). The rats weighed on average 254g at time of surgery and no significant changes in weight were detected between groups during the experiment. No animals were lost during the study and all animals were euthanized at their designated endpoint by an overdose of inhaled isoflurane.

Prior to the DMM surgical procedure, rats were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg). Sustained release buprenorphine (Sublocade, ZooPharm, Laramie, WY), 1 mg/kg) was administered by subcutaneous injection. The surgical site was then shaved and cleansed using multiple chlorhexidine washes before coating with povidone-iodine. Surgery was initiated when rats were unresponsive to tail pinch tests. Forty-two rats underwent bilateral surgeries involving a sham surgical approach on the left knee and destabilization of the medial meniscus on the right knee [15; 16]. Six rats were euthanized to obtain 12 pristine knees and 6 rats were euthanized at each time point and for each treatment group to obtain 6 sham and 6 DMM knees.

Acetaminophen and naproxen were suspended in 1% carboxymethylcellulose (CMC) before dosing. Rats were treated with placebo (1% CMC), acetaminophen (60 mg/kg), or naproxen (8 mg/kg) by oral gavage twice-a-day (morning and late afternoon) from day 15 through day 35 after DMM surgery. The naproxen and acetaminophen doses were based on prior rat studies [13; 17; 18]. The dose of acetaminophen used (60 mg/kg) is the maximum, recommended daily dose of acetaminophen in humans (4,000 mg/day) and a prior study established that fracture healing was not impaired in female Sprague-Dawley rats dosed daily with either 60 or 300 mg/kg of acetaminophen [18]. The dose of naproxen used (8 mg/kg) is approximately half the recommended long-term human dose of 1,000 mg/day and was effective in prior rat studies associated with arthritis [13; 17].

Six rats were euthanized 2 weeks after surgery to obtain baseline measurements of sham and DMM effects on proximal tibia articular cartilage. Six rats from each treatment group were euthanized at 5 and 7 weeks after surgery to measure drug treatment effects on proximal tibia articular cartilage and subchondral bone. The 5 and 7 week time points correspond to the end of 3 weeks of drug treatment and 2 weeks after drug treatment cessation, respectively.

Contrast-enhanced μCT analysis

Proximal tibias from the rats underwent μCT imaging. After resection, the specimens were fixed for 7 days in 10% formalin and stored in 70% ethanol at 4°C. Specimens were scanned using a Bruker Skyscan 1275 system (Bruker Corp., Billerica, MA). The μCT scan settings for unstained tibia were 70 kV, 142 μA, frame averaging of 4, rotation step of 0.4, and a 12 μm image pixel size, with a 1 mm thick aluminum filter. After the initial scan, each specimen was stained in 1% phosphotungstic acid (PTA; Sigma Aldrich Corp., St. Louis, MO) for 24 hours to visualize articular cartilage and then scanned a second time [19]. For the

PTA stained specimens, scan parameters were 100kV, 100 μA, frame averaging of 4, rotation step of 0.4, and a 12 μm image pixel size, with a 1 mm thick copper filter. The scan data were reconstructed using Bruker NRecon software and oriented into a standard alignment using DataViewer for later analysis.

Subchondral bone was analyzed using reconstructed images of pre-stained specimens that had been blinded for treatment. The subchondral bone volume was measured using the pre-contrast imaging data and was defined as the tissue between the articular cartilage and growth plate of the tibia. Medial subchondral bone volumes were selected from the center of each joint. Subchondral bone volumes were analyzed using CTAn (Bruker Corp., Billerica, MA) to determine the tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV), and trabecular thickness (TbTh).

Reconstructed images from before (pseudo-colored red) and after 1% PTA staining (pseudo-colored green) were aligned using the Register function of AnalyzePro software (AnalyzeDirect Inc., Overland Park, KS). Co-registration of the pre- and post-staining images enabled ready visualization of the proximal tibial cartilage (Figure S-1A). Cartilage depth was measured from the apparent tidemark to the outermost edge of the PTA-stained articular cartilage at three evenly spaced locations along the medial and along the lateral aspects of the proximal tibia (Figure S-1B). Measurement locations corresponded to the zones within the medial and lateral aspects of the tibia that were evaluated for histological OARSI scoring (see below). Specimens were blinded for treatment prior to analysis.

Histology and Histomorphometry

After μCT imaging, specimens were decalcified, paraffin embedded, and sectioned in the coronal plane (5 μm thick). Histological sections were stained with safranin-o stain and fast-green [20; 21]. Sections were viewed and digital images collected using an Olympus BX53 microscope and DP73 camera. Osteoarthritis severity was assessed using the Osteoarthritis Research Society International (OARSI) scoring system [15; 22]. Scores ranged from 0 (normal) to 5 (vertical clefts and erosion to the calcified cartilage extending greater than 75% of the articular cartilage surface) and were summed for the three zones (0-15) of the medial and for the lateral aspects of the knee [15; 22]. Cartilage area was measured using Osteomeasure software by manually tracing the remaining boundaries of the articular cartilage matrix (OsteoMetrics Inc., Decatur, GA). Osteophyte formation was also measured using the Osteomeasure software. Samples were scored from 0-4 based on osteophyte length (0 <200 μm, 1 = 200-299 μm, 2 = 300-399 μm, 3 = 400-499 μm, 4 > 500 μm) [22]. The absolute lengths of osteophytes for each sample were also compared [22]. Specimens were blinded for treatment prior to analysis.

Statistics

Six animals were used for each time point and treatment group based on a power analysis to detect a 50% difference in mean values at an $\alpha < 0.05$ and $ß > 0.8$ and using mean differences in cartilage changes reported in previous rodent DMM studies [15; 16]. Data were analyzed using one-way ANOVA and post-hoc Holm-Sidak corrected t-tests using Sigma Stat 4.0 (Systat Software Inc., San Jose, CA). Trabecular thickness data failed Shapiro Wilk

normality test and were analyzed using a Kruskal-Wallis One Way ANOVA on RANKS with a Dunn's multiple comparison post-hoc test. Initial statistical significance was set at $p < 0.05$.

Results

Thirty-six rats were necropsied to assess any drug effects on intestinal tract inflammation (12 for each treatment group, 6 for each time point). Inflamed intestine and colon were observed in 5 of 6 naproxen-treated rats at 5 weeks after surgery and within 2 hours after their final naproxen dosing. In the acetaminophen-treated rats, 1 of 6 rats had an inflamed intestinal tract at 5 weeks after surgery. In the placebo-treated rats, 1 of 6 rats had an inflamed intestinal tract at 5 weeks after surgery. At 7 weeks after DMM surgery (2 weeks after drug withdrawal), there were no signs of intestinal inflammation in any rat.

Tibias were resected, fixed, scanned by μCT, and then processed for histological examination. Representative histological images of the medial tibia plateau cartilage stained with safranin-O and fast green stained are shown in Figures 1 and S-2. Representative pre-contrast, post-contrast, and pseudo-colored combined images from the μCT analysis of 7 week post-surgery specimens are shown in Figures 3 and S-3. OARSI scores, cartilage area, cartilage depth, subchondral bone, and osteophyte measurements are summarized in Figures 2 and S-4, and Tables S-1 through S5.

As expected, histological observation of the pristine specimens showed a low, mean OARSI score of 1.9 and the articular cartilage appeared well defined with abundant glycosaminoglycans based on the intense safranin-O staining (Figure 1A). In contrast, 2 weeks after surgeries, both the sham (Figure 1B) and DMM (Figure 1C) treated rat specimens exhibited early indications of OA including decreased safranin-O staining intensity and changes in chondrocyte morphology. At 5 weeks after surgery (3 weeks of drug treatment), placebo treated rat specimens (Figure 1D) appeared to have minimal articular cartilage consistent with development of OA. Comparatively, acetaminophen (Figure 1E) and naproxen (Figure 1F) treated rat specimens still had evident articular cartilage. At 7 weeks after surgery (2 weeks after drug withdrawal), little or no articular cartilage was evident in the placebo treated rats though some islands of apparent fibrocartilage were evident (Figure 1G). The acetaminophen (Figure 1H) treated rat specimens also exhibited indications of severe OA with the near or complete absence of articular cartilage 2 weeks after drug withdrawal. Unexpectedly, 2 weeks after drug withdrawal, articular cartilage was still evident in the naproxen (Figure 1I) treated rat specimens, though cartilage fissures were visible. Lower magnification of these images is shown in Figure S-2.

At 2 weeks after DMM or sham surgery, proximal tibia medial and lateral cartilage was compared to that from pristine knees (Figure 2, and Table S-1). The OARSI score 2 weeks after DMM surgery for the proximal tibia medial cartilage was, as expected, significantly higher than the OARSI score from pristine tibia ($p<0.001$). No additional significant differences were detected at 2 weeks after the DMM or sham surgery for the OARSI scores, cartilage area or cartilage depth between the medial or lateral values from the pristine,

DMM, or sham specimens. Cartilage depth was also measured by comparing pre- and post-contrast μCT images of each knee (Figure S-3).

The OARSI scores, cartilage area, and cartilage depth values at 5 weeks after DMM surgery were compared (Figure 2 and Table S-2). Naproxen DMM specimens had a statistically lower medial OARSI score than the placebo DMM specimens ($p=0.025$). There was no statistical difference in medial OARSI scores between acetaminophen and naproxen ($p=0.079$) or placebo and acetaminophen ($p=0.444$) specimens. Medial cartilage area at 5 weeks was not significantly different between groups ($p=0.056$). However, medial cartilage depth was significantly greater in the naproxen and acetaminophen DMM groups as compared to the placebo DMM group ($p=0.005$ and $p<0.001$, respectively).

At 7 weeks after the DMM surgical procedures, naproxen DMM specimens had a statistically lower medial OARSI score than the placebo DMM ($p=0.024$ vs. placebo) and acetaminophen DMM groups ($p=0.003$ vs. acetaminophen, (Figure 2 and Table S-3). There was no statistical difference in medial OARSI scores between placebo DMM and acetaminophen DMM ($p=0.224$) samples. Medial cartilage area was significantly greater for naproxen DMM samples ($p=0.024$), compared to acetaminophen DMM samples (Figure 2 and Table S-3). There was no statistical difference in medial cartilage area between placebo DMM and naproxen DMM ($p=0.116$) or placebo DMM and acetaminophen DMM ($p=0.323$) samples. Cartilage depth was also measured by comparing pre- and post-contrast μCT images of each knee (Figure 3). Medial cartilage depth was significantly greater in the naproxen DMM group as compared to the acetaminophen group ($p=0.004$) but only approached being significantly greater than the placebo DMM group ($p=0.066$).

No significant differences were detected when comparing data from the proximal tibia lateral cartilage of the DMM operated knees at 5 or 7 weeks (Figure 2 and Tables S-2 and S-3). No significant differences were detected when comparing OARSI scores or cartilage area from the proximal tibia medial or lateral cartilage specimens of the sham operated knees at 5 or 7 weeks (Tables S-4 and S-5). However, medial cartilage depth in the sham surgery knees of the placebo treated rat specimens was significantly less than that from the acetaminophen or naproxen treated rat specimens at 5 weeks after surgery.

Medial subchondral bone structure was analyzed to determine whether subchondral bone structure was affected by acetaminophen or naproxen treatment. As shown in Table S-6, both acetaminophen and naproxen appeared to prevent bone loss at 5 weeks after the DMM surgery. The medial subchondral bone volume (BV) and relative bone volume (BV/TV) were significantly less in the placebo treated rat specimens than in the acetaminophen and naproxen treated rats at 5 weeks, while trabecular thickness (TbTh) was reduced in the placebo treated rat specimens as compared to the naproxen treated rat specimens. At 7 weeks after DMM and 2 weeks after cessation of acetaminophen and naproxen treatment, BV and BV/TV were significantly less in the placebo and acetaminophen treated rat specimens as compared to the naproxen treated rat specimens, though TbTh was only reduced in the placebo treated rat specimens when compared to the naproxen treated rat specimens.

Osteophyte analyses found a significant increases in scores and osteophyte length for the DMM baseline group, compared to pristine ($p = 0.005$) and sham controls ($p = 0.005$; Figure S-4).

Discussion

The results of this study indicate that naproxen treatment can slow OA progression the rat DMM OA model. Specifically, specimens from naproxen treated rats maintained medial articular cartilage depth and subchondral bone and had lower OARSI scores at 5 and 7 weeks after DMM surgery and more cartilage area at 7 weeks after DMM surgery as compared to placebo treated rat specimens (Figure 2 and Tables S-2, S-3, and S-6). Acetaminophen treatment also appeared to prevent articular cartilage loss in the rats at 5 weeks after DMM surgery (Figure 2 and Table S-2). Interestingly, the positive effects of naproxen treatment on preventing articular cartilage loss after DMM surgery persisted after naproxen treatment was withdrawn (Figure 2 and Table S-3). In sharp contrast, withdrawal of acetaminophen treatment led to rapid loss of any remaining medial articular cartilage (Figure 2 and Table S-3). This observation suggests that unlike acetaminophen, naproxen may have some disease modifying effects that persist after drug withdrawal. The pharmacokinetics of naproxen and acetaminophen may be consistent with the persistent effects on naproxen on preventing articular cartilage loss after drug withdrawal. The naproxen dose used in the present study (8 mg/kg, twice a day) should produce serum naproxen concentrations greater than 40 μg/ml, well above the 7 μg/ml IC_{50} needed to inhibit cyclooxygenase [7; 23]. In humans, naproxen is rapidly transported into the synovial compartment from which the elimination $T_{1/2}$ is over 24 hours [24]. In contrast, acetaminophen is rapidly eliminated from rat plasma and human synovial fluid with a $T_{1/2}$ of approximately 1 hour [25; 26].

Similar to the findings here, other studies using animal models of OA found that naproxen treatment generally restricted OA progression. In early studies, naproxen treatment reduced hind paw bone and cartilage erosion in the rat model of Freund's adjuvant-induced arthritis [17; 27]. In a rabbit model of *Staphylococcus aureus* induced arthritis, antibiotic treatment with naproxen reduced GAG loss by 50% as compared to antibiotic treatment alone [28]. Similarly, in a destabilized canine knee model of OA, naproxen treatment reduced loss of cartilage proteoglycan and reduced MMP activity [29]. In contrast, when inactivated *Mycobacterium tuberculosis* was directly injected in rat knees to induce arthritis, naproxen inhibited bone loss at the inflamed knee joint, but naproxen treatment caused greater GAG loss in the patellar tendon [30].

Though the primary pharmacological effect of NSAID administration is inhibition of COX-1 and COX-2 [4], NSAID effects on cartilage biology appear to extend beyond inhibition of cyclooxygenase and vary from one drug to another [31]. For instance, Palmonski and Brandt found that the non-selective NSAIDs indomethacin and sulindac sulfoxide had no effect on glycosaminoglycan (GAG) synthesis, while ibuprofen, fenoprofen, and salicylate inhibited (GAG) synthesis, and conversely, that benoxaprofen increased GAG synthesis in organ cultures of canine articular cartilage [32; 33]. In a rat in vivo model of OA, celecoxib treatment appeared to reduce OA progression while indomethacin and ibuprofen appeared to enhance

OA progression [34]. Conversely, genetic ablation of COX-1 or COX-2 had no effect on OA progression in articular cartilage in mice [35]. These varied findings indicate that ability of an NSAID to affect OA progression is drug specific and may be independent of the ability of each NSAID to inhibit COX-1 or COX-2.

Whether naproxen would have protective effects against OA induced cartilage loss would be difficult to predict based on cell culture studies. In micromass cultures, naproxen inhibited IL-1ß induction of MMP1, MMP13, and ADAMTS5, consistent with the protective effects of naproxen on OA related cartilage degradation noted in the present study [36]. In human MSCs cultured in chondrogenic media containing insulin and TGFß$_3$, naproxen treatment inhibited expression of the matrix degradation enzymes MMP13 and ADAMTS5 but also inhibited the expression of multiple cartilage matrix genes [37]. Naproxen induced type X collagen expression in cultures of human MSCs from normal and OA donors, which if operable within the context of articular cartilage would promote articular cartilage degradation [38]. Based on the results presented here and the studies noted above, the protective effects of naproxen may involve targeting processes in other tissues, such as the synovium, rather than having a direct protective effect in chondrocytes [39]. A limitation of our study was that we did not investigate the effects of naproxen and other anti-inflammatory drugs on DMM-induced synovitis. Our PTA-enhanced cartilage imaging protocol precluded us from leaving the joint intact, and thus we did not address the role of naproxen on the synovium.

Acetaminophen is also the recommended therapeutic for treating OA pain and in previous clinical studies, naproxen and acetaminophen had similar efficacy in reducing pain and improving function in OA patients [40-43]. The acetaminophen dose used in this study (60 mg/kg) can produce analgesia in rats since a previous study found that the same acetaminophen dose reduced bone fracture pain in a rat model [44]. However, acetaminophen does not target COX-1 or COX-2 [45; 46]. Van der Kraan et al. found that 200 mg/kg daily acetaminophen administration reduced serum sulfate levels which decreased patellar GAG content in male Wistar rats [47]. We did not quantify GAG content in our study. However, we noted near complete loss of safranin-O staining of GAG in the articular cartilage by 2 weeks after DMM surgery (Figures 1 and S-2). Neither acetaminophen nor naproxen treatment restored safranin-O staining in this study. Brandt and Albrecht noted that naproxen at the therapeutic dose (30 μg/ml) had no effect on GAG synthesis in organ cultures of canine articular cartilage, whereas Dingle et al. noted that naproxen failed to prevent IL-1α induced loss of GAG in organ cultures of porcine articular cartilage [48; 49].

The sustained effects of naproxen after treatment cessation on better preservation of articular cartilage is intriguing but the mechanism is unknown. At 5 weeks after DMM surgery, articular cartilage and subchondral bone values were similar between the naproxen and acetaminophen treatment groups (Tables S-2 and S-6). By 7 weeks after DMM surgery, articular cartilage values and subchondral bone volume for the acetaminophen treated rats were equivalent to placebo values, while the naproxen values remained significantly better (Tables S-3 and S-6). This suggests that simply preserving cartilage and bone tissues at 5 weeks was not sufficient to account for better values at 7 weeks. As noted above, chondrocyte and cartilage in vitro studies suggest that inhibited synthesis of matrix

degradation enzymes could account for the sustained preservation of articular cartilage in the naproxen treated rats. Additional experiments to measure reductions in the levels of MMP and other catabolic enzymes in the naproxen treated rat knee joints would be needed to confirm this potential mechanism.

Another possibility is that preservation of subchondral bone during naproxen treatment helps delay articular cartilage erosion following cessation of naproxen treatment. The DMM model is associated with greater osteoclast activity and bone loss in mouse and rat subchondral bone, as would occur during OA pathogenesis [16; 50]. Previous studies found that naproxen treatment can reduce bone loss in rabbits and ovariectomized rats [51; 52], in support of a potential mechanism for delaying articular cartilage erosion. Conversely, abnormally rapid loss of articular cartilage and subchondral bone after cessation of acetaminophen treatment may underlie the observed differences between acetaminophen and naproxen treatment withdrawal. Additional research is needed to understand the effects of naproxen, acetaminophen, and other analgesics on cartilage and bone biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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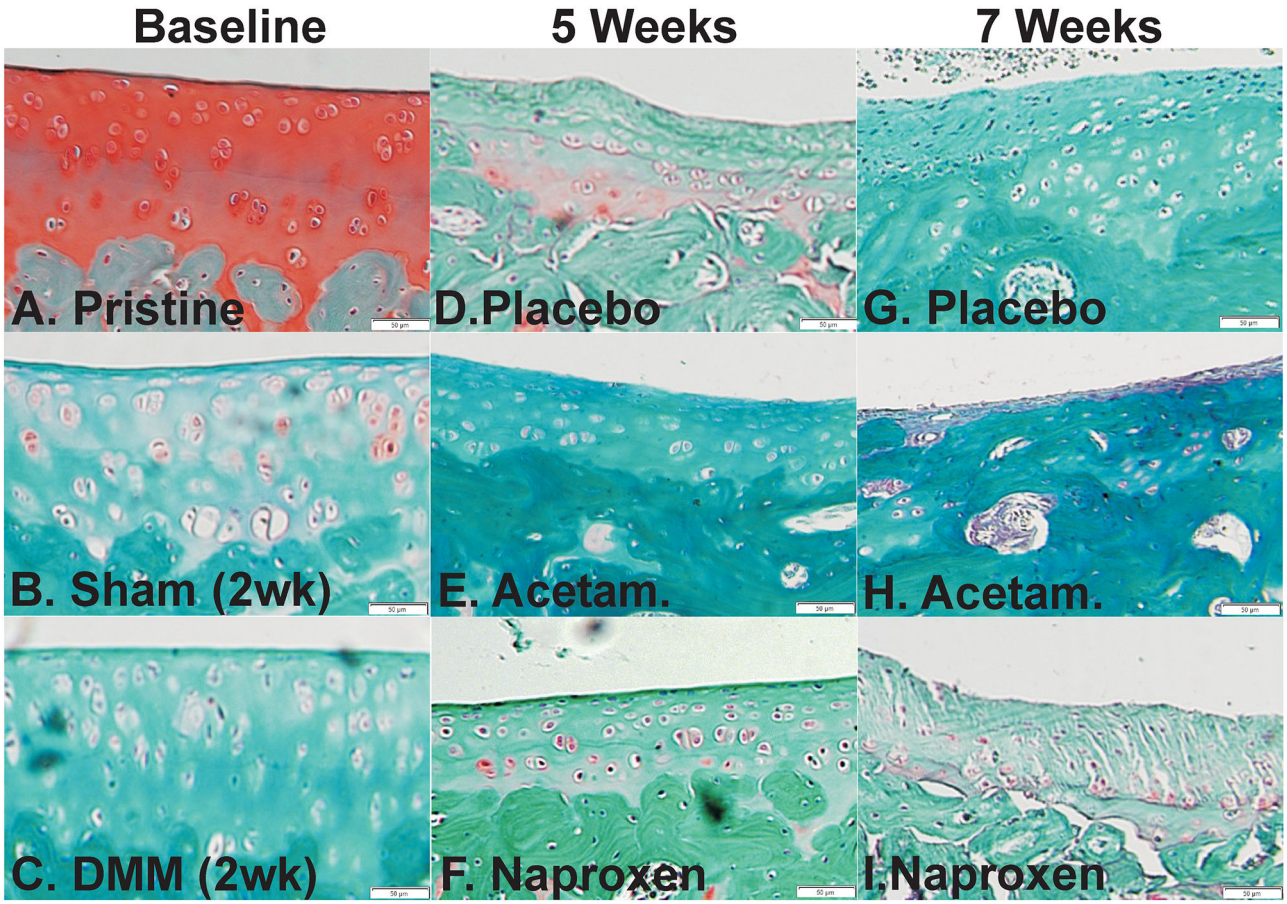

Figure 1.

Histological changes in the medial tibia cartilage following surgery and drug treatment. Shown are histology sections stained with safranin-O and fast green from pristine (A), baseline (2 weeks after surgery) sham (B), and baseline DMM (C) rat specimens. Histological sections from placebo (D, G), acetaminophen (E, H), and naproxen (F, I) treated rats are shown from specimens collected at 5 (D-F) and 7 weeks (G-I) after DMM surgery. Scale bar is 50 μm.

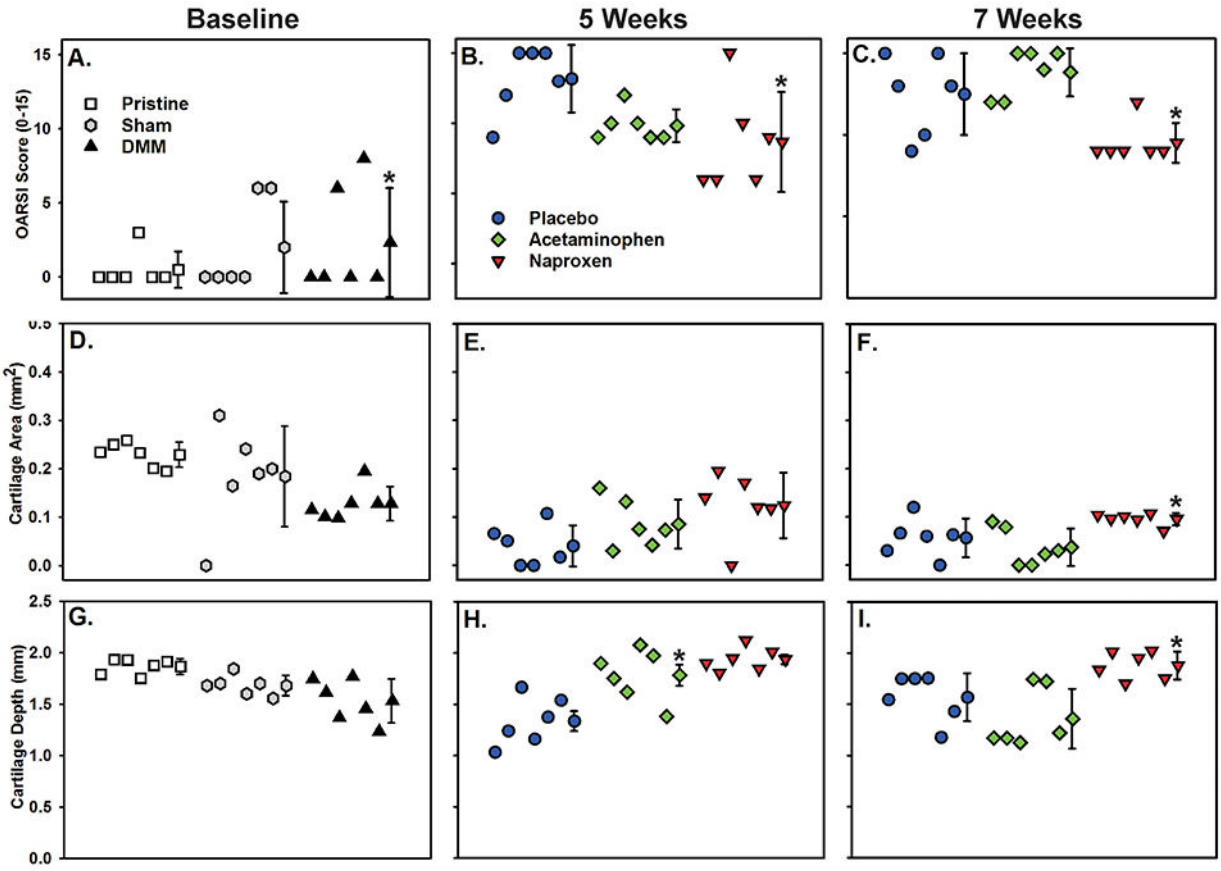

Figure 2.

Quantitative analysis of tibia articular cartilage. Plots detail OARSI scoring, cartilage area, and cartilage depth measurements taken from the medial aspect of tibias following DMM surgery at baseline (A, D, G), 5 weeks (B, E, H), and 7 weeks (C, F, I) after surgery (n=6 per group). Mean and standard deviation are shown after individual samples for each group. The Symbol legends are shown in panels A and B. Values significantly different than the pristine (panels A) or placebo control (panels B, C, F, and H) are indicated (*). Cartilage depth was also significantly greater in the 7 week naproxen group as compared to the acetaminophen group (panel I, *). See Tables S-2 and S-3 for numerical values.

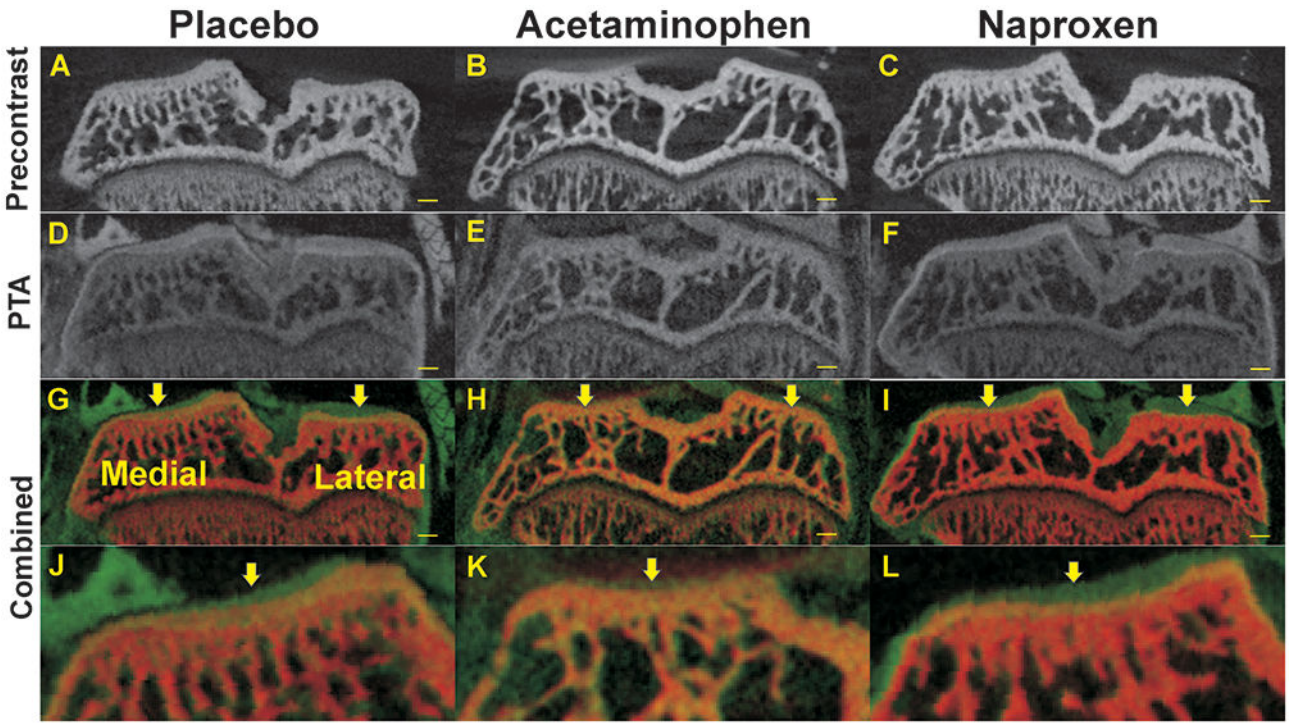

Figure 3.

Radiographic changes in tibia plateau cartilage and subchondral bone at 7 weeks after DMM surgery. Shown are pre-contrast (A-C), PTA post-contrast (D-F), and co-registered μCT images (combined, G-I) from rats treated with placebo (A, D, G, J), acetaminophen (B, E, H, K), or naproxen (C, F, I, L). Magnified views of the medial tibia plateaus are shown (J, K, L). Arrows indicate where articular cartilage depth was measured for the medial and lateral aspects of the tibia. Scale bar is 1 mm.