Inhibition of COX-2 by celecoxib in the canine groove model of osteoarthritis

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Objective. In vitro studies showed a beneficial effect of celecoxib on proteoglycan turnover and content of osteoarthritic cartilage. In the present study we evaluated whether these favourable effects of celecoxib could also be demonstrated in vivo. 

Methods. In 24 Beagle dogs, osteoarthritis (OA) was induced in one knee according to the groove model. The animals were divided into three groups and received oral placebo or 100 or 200 mg celecoxib daily, starting directly after surgery. After 15 weeks joint tissue from all dogs was analysed.

Results. Induction of OA resulted in macroscopic and histological damage of cartilage, changes in cartilage proteoglycan turnover, loss of cartilage matrix proteoglycans and slight synovial inflammation, all characteristic of early OA. Surprisingly, none of the parameters was significantly changed upon celecoxib treatment. Synovial fluid prostaglandin E2 levels were dose-dependently diminished by celecoxib, demonstrating that the celecoxib had reached the joint in sufficient amounts. Using an in vitro setup, canine cartilage under degenerative conditions was favourably influenced by celecoxib, demonstrating that canine cartilage is sensitive to celecoxib.

Conclusion. The present study showed a chondroneutral effect of celecoxib on the characteristics of experimentally induced OA in vivo, in contrast to the observed beneficial effect in vitro. It could be that celecoxib had been beneficial to degenerated cartilage in vivo but that these effects were counteracted by increased loading of the affected joint and the associated progression of OA, occurring because of the well-known analgesic effects of celecoxib.

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs in the treatment of osteoarthritis (OA) [1]. The clinical efficacy of NSAIDs is primarily related to the inhibition of cyclooxygenase 2 (COX-2), whereas much of the toxicity, particularly in the gastrointestinal system, is related to COX-1 inhibition [2]. This has led to selective COX-2 inhibitors that specifically block COX-2 with similar efficacy and less toxicity [3]. These COX-2-specific drugs have been used clinically for the past few years in the treatment of (osteo)arthritis and other conditions [3, 4]. However, more recently it has appeared that selective COX-2 inhibition also has its dark side and that it might increase the risk of cardiovascular diseases [5–7]. In this respect caution and further study are needed.

Another problem with NSAIDs might be their direct (adverse) effects on cartilage. These effects cannot be studied easily in clinical trials and therefore they are generally ignored in clinical practice because (intrinsic) cartilage changes, catabolic and anabolic, are rather slow processes in OA. In addition, beneficial effects of NSAIDs on inflammation mask possible direct adverse effects on cartilage.

Direct effects of NSAIDs on cartilage may be important specifically in prolonged treatment of joint disease in which inflammation is only mild and secondary, as in OA. Data on direct effects of conventional NSAIDs on cartilage are numerous, but results are far from conclusive [8, 9]. Two frequently used NSAIDs that have been studied regarding their direct effects on cartilage are naproxen and indomethacin. Studies on the influence of naproxen show conflicting results; there are signs that naproxen suppresses cartilage proteoglycan synthesis in vitro [9, 10], but other studies show suppression of proteoglycan degradation [11, 12]. Indomethacin shows principally negative effects on the biochemical parameters of cartilage in in vitro [9, 10] and animal studies [13, 14], but there are also studies that did not find any effect of indomethacin on cartilage [15, 16]. Thus, although NSAIDs may be very useful regarding symptoms in OA, reducing pain and inflammation, they may interfere directly with the processes of cartilage degeneration and regeneration. These direct effects should be considered in addition to gastrointestinal and cardiovascular effects when prescribing NSAIDs in clinical practice.

With respect to the selective COX-2 inhibitors, data are limited. We recently showed a beneficial effect of celecoxib in normal cartilage under the influence of IL-1 and TNF-α; on normal healthy cartilage no effects were observed [17]. Findings by El Hajjaji et al. [18] showed that celecoxib in vivo was able to increase proteoglycan synthesis and to diminish proteoglycan release of OA cartilage obtained at joint replacement surgery. Recent findings by our group confirmed these data and additionally demonstrated that, in vitro, celecoxib had a favourable effect on proteoglycan synthesis, retention, release and content of both degenerated (preclinical) and (late-stage) human OA cartilage [19]. However, COX-2 has also been reported to be involved in normal physiology, including bone healing [20, 21].

The lack of in vivo data from animal models of OA urged us to study selective COX-2 inhibition in such a model. The groove model of OA [22] has features representative of human OA [23].
The model is distinctive in that the degenerative changes are progressive while synovial inflammation diminishes over time [24]. Because of this, evaluation of direct effects of medication on cartilage is less hampered by a possible anti-inflammatory effect of treatment. Additionally the model is distinctive because there is no permanent trigger causing joint damage, making the model more sensitive to treatment. A permanent trigger for joint damage, such as the joint instability used in the anterior cruciate ligament transection (ACLT) model, will counteract the possible beneficial effects of treatment. Assuming that cartilage repair is possible [25–27], the trigger, intrinsic to the cartilage damage itself, can be eliminated by treatment. Therefore, we chose the canine groove model of OA to study the effect of celecoxib on OA cartilage in vivo.

Materials and methods

Animals

Female Beagle dogs, 24 in number, mean age 1.8 ± 0.1 yr, weighing 10–15 kg, were obtained from the animal laboratory of Utrecht University in the Netherlands. They were housed in groups of two or three dogs per pen, and were let out on a patio in large groups for at least 2 h daily. They were fed a standard diet and had water ad libitum. The Utrecht University Medical Ethical Committee for animal studies approved the study.

The groove model

After induction with nesdonal, the dogs were anaesthetized with halothane in a mixture of oxygen and nitrous oxide delivered endotracheally. Surgery was carried out through a 2–2.5 cm medial incision close to the patellar ligament in the right knee. Care was taken to prevent bleeding and soft tissue damage as much as possible. Cartilage of the lateral and medial condyle was damaged with a Kirschner wire (1.5 mm diameter) that was bent 90° 0.5 mm from the tip. This ensured that depth of the grooves was restricted to around 0.5 mm. In utmost flexion, 10 longitudinal and diagonal grooves were made on the weight-bearing parts of the femoral condyles without damaging the subchondral bone [22–24]. The latter was confirmed by histology at the end of the experiment. Macroscopic evaluation after killing of the animals showed similar groove patterns in all affected knees. The tibial plateau was left untouched. After surgery, synovium, fasciae and skin were sutured. The contralateral unoperated knee served as a control. All the animals received analgesics (buprenorphine 0.01 mg/kg) and antibiotics (amoxicillin 400 mg/kg) during the first 3 days after surgery. Starting 2 days after surgery, the dogs were let out daily on the patio. To ensure (intensified) mechanical loading of the affected knee, the dogs were forced to load the experimental joint intermittently by fixing the contralateral limb to the trunk for approximately 4 h per day, 3 days per week, for 15 weeks. This is less than 10% of the total loading time of the affected joint. Thus, the control limb was not weight-bearing for less than 10% of the time. We have demonstrated that the control limbs of these animals did not differ from those of animals from which the limbs were not fixed to the trunk [23].

Treatment

The dogs were divided into three groups of eight animals each. Celecoxib (Celebrex; Pfizer, USA) was given once daily orally in two doses; one group received 100 mg/day, the second group received 200 mg/day, and the third group received a placebo. Treatment was started the day after surgery. The choice of these doses was based on previous studies that focused on the plasma kinetics of celecoxib in Beagle dogs [28, 29]. The treatment compliance was 100%.

Evaluations

Severity of OA was evaluated 15 weeks after surgery. At the end of the experiment, the dogs were euthanized with an intravenous injection of euthesate (Na-pentobarbital). Both hind limbs were amputated and synovium and cartilage were collected and processed within 2 h. Procedures were carried out under laminar flow conditions.

Synovial tissue analysis

Macroscopic synovial inflammation was evaluated on digital high-resolution photographs of synovium, by two observers unaware of the source of the photographs. Severity of inflammation was graded from 0 to 2 for colour, angiogenesis and fibrillation: 0 = none, 1 = slightly, 2 = strong. The sum of these three individual scores averaged for the two observers (a maximum of 6) was used as the representative score for each joint and used for statistical analysis.

Three infrapatellar synovial tissue samples for each joint (medial, middle and lateral) were fixed in 4% phosphate-buffered formalin (pH 7.0) and embedded in paraffin. Deparaffined sections were stained with haematoxylin–eosin. Histological sections were examined separately in random order and independently by two observers unaware of the source of the synovium. To determine the degree of inflammation, the slightly modified [30] criteria described by Goldenberg and Cohen [31] was used. For assessing the overall grade, the scores of the three specimen from each knee and of the two observers were averaged (maximum of 10). This score was used as the representative score for each joint and was used for statistical analysis.

Cartilage analysis

Macroscopic cartilage damage was evaluated on digital high-resolution photographs of the tibia and femur by two observers unaware of the source of the photographs. Severity of cartilage damage of the femoral condyle was graded from 0 to 4: 0 = smooth surface, 1 = slightly fibrillated, 2 = fibrillated with shallow grooves, 3 = deep sharp grooves, 4 = deep sharp grooves with surrounding damage. Grading of cartilage damage of the tibial plateau was comparable: 0 = smooth surface, 1 = roughened, 2 = slightly fibrillated, 3 = fibrillated, 4 = damaged. Scores of the two observers were averaged (maximum of 4). This score was used as the representative score for each photograph and was used for statistical analysis.

Cartilage samples for histological and biochemical analyses were obtained from predetermined locations on the weight-bearing areas of the femoral condyles and the tibial plateau of the experimental and control joints. Locations were identically paired with the same location in the contralateral joint [23]. Cartilage was cut as thick as possible while excluding the underlying bone, and subsequently samples were cut into full-thickness cubes weighing 3–10 mg (accuracy 0.1 mg).

For histology, four samples from the tibial plateau and four from the femoral condyles from each knee were fixed in 4% phosphate-buffered formalin containing 2% sucrose (pH 7.0). Cartilage degeneration was evaluated in sections stained with safranin O-fast green iron haematoxylin by light microscopy using the slightly modified [32] criteria of Mankin [33]. Specimens were graded in random order by two observers unaware of the source of the cartilage. For assessing the overall grade, the scores of the four specimens from each knee surface and of the two observers were averaged (maximum of 11). This score of each joint surface was used as the representative score and used for statistical analysis.

For biochemical analysis, cartilage samples were cultured individually in 96-well culture plates (Nunclon®, Denmark) in
Proteoglycan synthesis. As a measure of proteoglycan synthesis, the rate of sulphate incorporation was determined ex vivo [34]. After 4 h of labelling with $^{35}$SO$_2^-$, the cartilage samples were washed with cold phosphate-buffered saline and digested with papain for 2 h at 65°C. Glicosaminoglycans (GAGs) were precipitated by addition of cetylpyridinium chloride (CPC), and GAGs labelled with $^{35}$SO$_2^-$ were measured by liquid scintillation analysis. Synthetic activity is expressed as mmol of sulphate incorporated per hour per gram wet weight of the cartilage (nmol/h/g).

Proteoglycan retention and release. As a measure of the retention of newly synthesized proteoglycans, the release of $^{35}$SO$_2^-$-labelled proteoglycans in the medium was determined. After labelling, the cartilage samples were rinsed three times for 45 min in 1.5 ml culture medium and then incubated in 200 μl fresh culture medium without sulphate label for 3 days. GAGs were precipitated from the medium and were stained with Alcian blue dye solution, as described previously [34]. The $^{35}$SO$_2^-$-labelled GAGs were measured by liquid scintillation analysis and release was normalized to the specific activity of the medium and the wet weight of the explants. The release of newly formed proteoglycans is expressed as percentage release of newly formed proteoglycans in the 3 days (% new proteoglycan release). For the total release of proteoglycans, Alcian blue staining of the medium was quantified photometrically with chondroitin sulphate (Sigma C4384) as a reference. The total amount of GAGs released (blue staining) is expressed as a percentage of the original tissue content (% GAG release).

Proteoglycan content. As a measure of proteoglycan content of the cartilage samples, the amount of GAG was determined as described previously [34]. The GAGs in the papain digest of cartilage samples were precipitated and stained with Alcian blue as described above. Values were normalized to the wet weight of the cartilage explants (mg/g).

Prostaglandin $E_2$ determination

Prostaglandin $E_2$ was determined in thawed (frozen at −80°C) synovial fluid that had been taken after the joints had been opened, by enzyme immunoassay (EIA; Amersham Biosciences) and expressed as pg/ml.

In vitro analyses

In addition, we performed in vitro experiments to see whether ‘human’ celecoxib is able to modify degenerative changes of canine cartilage. Canine synovial tissue, obtained from knees with experimentally induced OA, was cultured for 3 days [35]. Cell-free supernatants were added to healthy canine hip cartilage at a concentration of 50% v/v, resulting in OA-characteristic changes in proteoglycan turnover and content. These cultures were performed in the absence and presence of celecoxib (10 μM) to study whether celecoxib could reverse these effects as previously reported for human degenerated cartilage [17, 19].

Calculations and statistics

Mean values ± S.E.M. (n = 8 animals) of each joint for femoral and tibial cartilage separately are presented. Paired Student’s t-test was used to compare data of the experimental and contralateral control joints within each group. The unpaired t-test was used to analyse differences between the three treatment groups (absolute values of the control and experimental joints separately and the change in the experimental compared with the control joints). P values less than 0.05 were considered statistically significant.

Results

Induction of OA in the groove model

Cartilage damage. Fifteen weeks after induction of experimental OA, the affected knees clearly showed macroscopic damage of the articular cartilage of femoral condyles in addition to the grooves that were surgically applied and still visible. No damage was found on the condylar cartilage of the control knees (for representative photographs see Fig. 1A vs B). Damage, although less pronounced, was also found on the tibial plateau of the experimental joints compared with the control joints (Fig. 1C vs D). On average, the macroscopic cartilage damage was significantly more severe in the experimental joints compared with that in the control joints (Fig. 1G, placebo; solid lines represent the femoral condyle and dashed lines represent the tibial plateau).

These macroscopic observations were confirmed by histological analysis. The average modified Mankin score of the cartilage degeneration in the experimental femoral condyles was mild but significantly higher compared with that of the contralateral control joints (Fig. 2G, placebo; solid line). The grooves were clearly visible, as depicted by a representative micrograph in Fig. 2A vs B. Although not surgically damaged, mild cartilage degradation of the experimental tibial plateau cartilage was also found in the placebo OA group when compared with the contralateral control joints (representative micrographs are given in Fig. 2C vs D). On average, histological damage of the tibial plateau was statistically significant different from that of control joints (Fig. 2G, placebo, dashed line).

Synovial inflammation. Macroscopically, the synovial tissue showed mild signs of inflammation in the experimental joints compared with the contralateral control joints. Figure 1E and F represents representative photographs; Fig. 1H (left) depicts the averages for the placebo group. This was confirmed by light microscopic examination of the synovial tissue (Fig. 2E vs F and H, respectively).

Proteoglycan turnover. Fifteen weeks after surgery, synthesis of proteoglycans of both femoral condyles as well as tibial plateaus was increased in the experimental joints compared with the contralateral control joints (Fig. 3A). This increased proteoglycan synthesis, characteristic of OA, is ineffective as the release of these newly formed proteoglycans was also increased in the experimental OA joints. Also, the release of the total amount of proteoglycans was enhanced as a result of the experimentally induced OA in both femoral condyle and tibial plateau cartilage (Fig. 3C). As result of the ineffective proteoglycan synthesis and enhanced proteoglycan release, the content of proteoglycans was significantly decreased in cartilage of the femoral condyles and tibial plateau (Fig. 3D). There was no statistically significant change in cartilage DNA content due to
induction of OA (OA 0.12 ± 0.01 vs control 0.15 ± 0.01 mg/g and 0.13 ± 0.00 vs 0.12 ± 0.01 mg/g, for femoral condyles and tibial plateau, respectively).

Effect of celecoxib treatment

One day after OA induction, celecoxib treatment was started. There were no clinical signs of discomfort, such as apathy, loss of appetite or weight loss, during the study. Although all dogs were active after the surgery, the celecoxib-treated dogs (100 mg and 200 mg) seemed to walk better and more when compared with the placebo group. Although this difference in walking pattern was noted independently by three observers (S.M., M.V. and F.L.) and discussed, this was not further quantified.

Cartilage damage. In all animals treated with celecoxib, both 100 and 200 mg, macroscopic cartilage damage was not different from that of the OA placebo groups. This applied both to the femoral condyles and to the tibial plateau, and whether considered in terms of absolute values or as changes (Fig. 1G).

Histological examination of the different groups confirmed the macroscopic impression and showed similar results for the celecoxib-treated groups and the placebo group (Fig. 2G).

Synovial inflammation. Synovial inflammation in the celecoxib-treated group when compared with the OA placebo group was not different based on macroscopic evaluation (Fig. 1H) and microscopic evaluation (Fig. 2H).

Proteoglycan turnover under the influence of celecoxib. Biochemical evaluation of proteoglycan turnover and content demonstrated that celecoxib at both concentrations had not resulted in a difference in these parameters of condylar and tibial plateau cartilage (Fig. 3A–D). Also DNA content was not changed by celecoxib (data not shown).

Prostaglandin E2 levels in synovial fluid. Because of the lack of any significant difference in the celecoxib-treated groups compared with the placebo OA group, it was doubtful whether celecoxib had entered the joint in sufficient amounts. Therefore, the synovial fluid samples were analysed for prostaglandin E2. In the synovial fluid of the placebo-treated group, we found increased levels of prostaglandin E2 (Fig. 4). In the group treated with 100 mg celecoxib the levels of prostaglandin E2 were much lower, although still elevated compared with controls. In the animals treated at 200 mg, prostaglandin E2 levels were even lower and not statistically different from those in control joints.
Interestingly, a decrease in synovial fluid prostaglandin E$_2$ levels was also observed in the contralateral control joints, although the difference was not statistically significant.

**In vitro effect of celecoxib on canine cartilage.** Because celecoxib did not demonstrate an effect on the articular cartilage, although apparently present in sufficient amounts, it was questionable whether celecoxib was able to influence canine cartilage. This was evaluated *in vitro*. Canine synovium tissue supernatants were used to induce degenerative changes in healthy canine cartilage in the presence or absence of celecoxib. Celecoxib was able to influence the (degenerated) cartilage beneficially (Fig. 5). The enhanced proteoglycan release was decreased to levels even below that of the control cartilage (not treated with synovium culture supernatants; Fig. 5A). Also, the proteoglycan content of canine cartilage was increased by celecoxib to levels of controls (Fig. 5B). Proteoglycan synthesis was only mildly increased, which is in accordance with the effects of celecoxib on proteoglycan synthesis in human degenerated cartilage [17, 19].

**Discussion**

To our knowledge, this study describes for the first time the *in vivo* effect of celecoxib on experimental OA cartilage, using the canine groove model of OA. Several recent *in vitro* studies showed that the selective COX-2 inhibitor celecoxib is able to improve and even restore disturbed cartilage turnover, both under inflammatory conditions [17] and under OA conditions [18, 19]. Normal healthy cartilage was not influenced by celecoxib in these studies [17, 19]. However, we could not confirm the positive *in vitro* results of celecoxib in our *in vivo* canine model of OA.

Increased synthesis of proteoglycans, decreased retention of these newly formed proteoglycans and increased release of proteoglycans combined with a decreased proteoglycan content, as found in the placebo-treated OA group, characteristic of OA, are comparable to previous studies performed with this model [23, 24] and therefore are not expected to be the cause of the absence of an effect of celecoxib.

There is evidence for polymorphism in the metabolism of celecoxib in Beagle dogs [28]. There are at least two populations of dogs, distinguished by their capacity to eliminate celecoxib from
plasma at either a fast or a slow rate after intravenous administration. About half of the 242 tested Beagles were of the fast phenotype and the other half were of the slow phenotype. There was also an equal distribution of the two phenotypes within males and females. The difference between the two populations was due to a difference in the rate of metabolism of celecoxib by the liver cytochrome P-450. We were not able to check our Beagle dogs for this polymorphism. As we used animals with the same genetic background, we assumed that they are either slow or fast metabolizers. Therefore the doses of celecoxib were chosen in such a way that at one of the two doses (100 or 200 mg) an effective concentration that was effective in inhibiting COX-2 activity. Also, the control joints of the celecoxib-treated animals showed lower prostaglandin E2 levels (although the difference was not statistically significant). This suggests that in the contralateral control joints some COX inhibition had also occurred as a result of the treatment. Nevertheless, despite functional COX(-2) inhibition in the joint, no effects on cartilage were found.

Because of a lack of effect despite the doses chosen, we questioned whether celecoxib reached the joint in an adequate concentration. Therefore, prostaglandin E2 levels in the synovial fluid at the end of the study were measured. Prostaglandin E2 is one of the main products formed by COX-2 and is an indirect measure of COX-2 activity. In the synovial fluid of the placebo-treated group, we found increased levels of prostaglandin E2. This corroborates the human OA condition, where increased levels of prostaglandin E2 in different joint tissues are also found [19, 36].

More interestingly, we found in the celecoxib-treated groups, dose-dependently, much lower levels of prostaglandin E2. This indirectly proves that celecoxib had entered the joints at a concentration that was effective in inhibiting COX-2 activity. Also, the control joints of the celecoxib-treated animals showed lower prostaglandin E2 levels (although the difference was not statistically significant). This suggests that in the contralateral control joints some COX inhibition had also occurred as a result of the treatment. Nevertheless, despite functional COX(-2) inhibition in the joint, no effects on cartilage were found.

This raises the question of whether canine articular chondrocytes are sensitive to ‘human’ celecoxib. It appeared that canine cartilage under the influence of catabolic mediators released by OA synovial tissue was beneficially influenced by celecoxib. These in vitro effects of celecoxib were comparable to results obtained with human cartilage in vitro exposed to celecoxib [17–19].

Thus, although celecoxib had reached the joint and COX-2 inhibition can be beneficial for canine cartilage under degenerative conditions, in our in vivo study no effects of celecoxib were observed. A possible explanation could be found in the different walking patterns observed in the different treatment groups. The celecoxib-treated dogs (both 100 mg and 200 mg) seemed to walk better and more when compared with the placebo group. This fits well with the analgesic effects of celecoxib. It could be...
that celecoxib had been beneficial to degenerated cartilage \textit{in vivo} but that these effects were counteracted by the increased loading of the affected joint and the associated progression of OA, because of the analgesic effects of celecoxib. The efficacy of celecoxib in reducing knee pain in patients with OA has been demonstrated in previous studies [37, 38]. Recently the effect of celecoxib vs placebo treatment was compared on clinical and gait variables in knee OA patients, focusing on the efficiency of the locomotor mechanism [39]. In this study, celecoxib treatment improved the efficiency of the locomotor mechanism significantly. Among the secondary outcome measures assessed, walking cadence was improved by celecoxib treatment and knee pain was significantly reduced.

Improved walking and loading of the animals treated with celecoxib might have resulted in undesired progression of OA in our experiments, counteracting the direct beneficial effects on cartilage. The absence of a net effect on cartilage may also explain the absence of a net effect on histochemically determined synovial inflammation, because, in our model, inflammation is very mild and depends primarily on cartilage damage [22, 23]. Increased loading of a joint is associated with OA pathology [40–43]. \textit{In vivo} animal experiments indicate that impulsive loading and joint instability results in bone or cartilage changes commonly associated with OA [40, 44, 45]. Severe OA rapidly developed in dogs following anterior cruciate ligament transection combined with dorsal root ganglionectomy [44]. Rapid development of OA was attributed to the increased ground reaction forces, which were 30% higher in these dogs compared with ACLT dogs with an additional sham procedure [46], a difference that may have resulted from the impaired ability of the dogs to experience knee instability and pain. However, the effects of training on cartilage are known to be ambivalent [47, 48]. Moderate exercise seems not to increase the risk of OA, and under certain circumstances even could prevent knee OA [48].

In this perspective, it might have been better to treat the placebo group also with an analgesic. However, this is difficult because gastrointestinal side-effects of conventional NSAIDs are much more severe in dogs than in humans [49]. Furthermore, conventional NSAIDs could have had an adverse direct effect on the cartilage [8, 50]. Opioids are also no option because they will cause

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**Fig. 4.** Synovial fluid prostaglandin E$_2$ levels in experimental OA with or without celecoxib treatment. Synovial fluid prostaglandin E$_2$ levels of control and experimental joints treated with placebo (diamonds), celecoxib 100 mg (squares) or celecoxib 200 mg (triangles). Mean values ($n=8$, $\pm$S.E.M.) are presented for animals treated with placebo, celecoxib 100 mg and celecoxib 200 mg. Statistically significant differences in the effects of celecoxib compared with untreated controls, calculated by non-parametric paired analysis ($P<0.05$) are marked with an asterisk.

**Fig. 5.** The \textit{in vitro} effect of celecoxib on canine cartilage; changes in proteoglycan turnover. Percentage total release of proteoglycans (A), proteoglycan content (B) and proteoglycan synthesis rate (C) are depicted. Mean values ($n=6$, $\pm$S.E.M.) are presented for cartilage cultured with 50% v/v experimental OA synovial tissue culture supernatants in the absence or presence of celecoxib (10 $\mu$M). Dashed lines indicate levels in healthy canine cartilage cultured alone. Asterisks indicate statistically significant ($P<0.05$) change induced by celecoxib compared with cartilage cultured in the presence of 50% synovium culture supernatant alone, as calculated by paired Student’s $t$-test.
interference with walking/joint loading activity. Buprenorphine treatment, which was used as a pain-killer for 3 days after surgery, led to loss of appetite. Paracetamol (acetaminophen) causes toxicity in dogs after repetitive administration of therapeutic doses [51, 52]. In this respect it is remarkable that the prolonged use of celecoxib showed no signs of side-effects. The veterinary market might in this respect offer a new application of this selective COX-2 inhibitor.

All together, this study showed no beneficial effect of celecoxib on the proteoglycan turnover of experimentally induced OA. It could be that celecoxib had been beneficial to degenerated cartilage in vivo but that these effects were counteracted by the increased loading of the affected joint and the associated progression of OA, because of the analgesic effects of celecoxib. To establish whether this concept is plausible further research using, for example, gait analyses is needed.

### Key messages

- The present study showed no effect of celecoxib on the characteristics of experimentally induced OA in vivo, in contrast to the observed beneficial effect in vitro.
- It could be that celecoxib was beneficial to degenerated cartilage in vivo but that these effects were counteracted by increased loading of the affected joint and associated progression of OA, because of the well-known analgesic effects of celecoxib.

### Acknowledgements

We thank Hans, Nico, Jannie, Hester, Aline and Maurits [Animal Facilities of UMC Utrecht (GDL)] for their support during this study. F.P.L. was supported by the Dutch Arthritis Association.

An unrestricted research grant was received from Pfizer.

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