OsteoArthritis and Cartilage (2005) **13,** 519–526

doi:10.1016/j.joca.2005.02.004

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Selective COX-2 inhibition is favorable to human early and late-stage osteoarthritic cartilage: a human *in vitro* study[1]

S. C. Mastbergen M.Sc*, J. W. Bijlsma M.D., Professor and F. P. Lafeber Ph.D.
Rheumatology & Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands

Summary

Objective: Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of osteoarthritis (OA). For the outcome of treatment the direct effects of NSAIDs on cartilage may be more important than indirect effects on inflammation, considered being secondary in OA. For clinical practice, it is relevant to study effects of NSAIDs on early stages of OA. Therefore we studied the direct effects of celecoxib on human degenerated OA cartilage and compared the effects with those on human healthy cartilage and human end-stage OA cartilage.

Methods: Degenerated, late-stage OA, and healthy human articular cartilage were exposed (7 days of culture) to celecoxib (0.1–10 μM). Changes in cartilage proteoglycan turnover (synthesis, retention, and release), proteoglycan content, prostaglandin E_2 (PGE_2) and nitric oxide (NO) production were determined.

Results: Both degenerated and established OA cartilage showed its characteristic changes in proteoglycan turnover (all $P < 0.05$). Celecoxib at 1 μM was able to increase synthesis of degenerated cartilage and normalize both releases of newly formed and resident proteoglycans. Importantly, 1 μM celecoxib influenced matrix integrity by enhancing proteoglycan content. Similar results were found for end-stage OA cartilage. Enhanced PGE_2 production in degenerative and OA cartilage could be decreased by celecoxib, whereas no effect on enhanced NO production was found. No significant effects of celecoxib on normal cartilage were found.

Discussion: Celecoxib, in a clinical relevant concentration, showed *in vitro* a significant beneficial effect, not only on late-stage OA but also on more early stages of OA, whereas healthy cartilage remained unaffected, suggesting chondroprotective properties of celecoxib in the treatment of degenerative joint disorders.

Key words: Human cartilage, Osteoarthritis, COX-2 inhibition.

Introduction

In osteoarthritis (OA) damage to cartilage is characterized by loss of matrix proteoglycans and damage of the collagen structure. Although OA is primarily an intrinsic process of the cartilage, secondary synovitis may trigger clinical symptoms and add to the final damage of the cartilage tissue and with that to joint damage in general[1–3].

Nonsteroidal anti-inflammatory drugs (NSAIDs) diminish inflammation, therefore NSAIDs may indirectly be beneficial to cartilage under inflammatory conditions although this has never been proven in clinical trials. Based on *in vitro* and animal studies, direct effects of NSAIDs on cartilage have frequently been reported[4–7]. Although different experimental setups have been used in different studies these studies clearly show that some of the used NSAIDs have direct adverse effects on cartilage, though beneficial and neutral effects have been reported as well[5–8]. These direct effects cannot be studied easily in clinical trials and therefore they are generally ignored in clinical practice. This is because these direct effects on cartilage are shaded by the effects of NSAIDs on inflammation and because (intrinsic) cartilage changes, catabolic and anabolic, are generally very slow processes in OA.

Direct effects of NSAIDs on cartilage may be important specifically in long-term treatment of joint disease in which inflammation is only mild and secondary as in OA. Thus, although NSAIDs may be very useful in reducing pain and inflammation in OA, when their direct effects are adverse, they may contribute to the process of cartilage degeneration by interfering with the intrinsic repair activity.

The anti-inflammatory effects of NSAIDs are mainly due to their ability to inhibit prostaglandin production by cyclooxygenase (COX) in a non-specific manner, thereby demonstrating efficacy but also toxicity[9–11]. The discovery of two isoforms of COX, COX-1 and COX-2, provided insight into the mechanism of action and toxicity of NSAIDs. Much has been learned about these isoenzymes since their discovery.

The discovery of COX-2 has spurred the development of drugs that could selectively inhibit COX-2, lacking the adverse effects of traditional NSAIDs. Celecoxib, as one of the first selective COX-2 inhibitors, has been shown to be an effective anti-inflammatory and analgesic drug in patients with rheumatoid arthritis and OA, comparable to that of traditional NSAIDs, such as naproxen, diclofenac and ibuprofen[12–14]. A significant reduction of gastrointestinal adverse events with selective COX-2 inhibitors compared to non-selective NSAIDS has been demonstrated frequently.

In the treatment of OA, as explained above, it remains at least as important to know the direct effects of these drugs on cartilage. This is specifically important because COX-2 is found to be expressed in OA tissues[15,16]. Both in

[1]Supported by a grant from Pfizer.
*Address correspondence and reprint requests to: Simon C. Mastbergen, Rheumatology & Clinical Immunology, University Medical Center Utrecht, Room F02.127, P.O. Box 85500, 3508GA Utrecht, The Netherlands. Fax: 31-30-2523741; E-mail: s.mastbergen@azu.nl
Received 19 August 2004; revision accepted 6 February 2005.

chondrocytes and synovial cells of OA joints elevated levels of COX-2 have been found. Also elevated levels of prostaglandin E_2 (PGE_2) have been found in OA cartilage, indirectly demonstrating a role for COX-2 in diseased cartilage[17]. Most recently, the expression of COX-2 and PGE_2 in OA meniscus, synovial membrane, osteophytic fibrocartilage and in the articular OA cartilage has been described[15]. Furthermore, the latter study showed an increased proteoglycan degradation that correlated with COX-2 protein expression and PGE_2 production by the synovial membrane. In a previous study of our group we showed a beneficial effect of COX-2 inhibition in interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) treated cartilage[18]. Because these mediators play a role in cartilage damage in OA, this finding indirectly indicates a possible role of COX-2, in the process of cartilage degeneration and the attempt to repair, as seen in OA. Recent findings by El Hajjaji *et al.*[19] showed that celecoxib was able to increase proteoglycan synthesis and was able to diminish proteoglycan release of OA cartilage obtained at joint replacement surgery. However, this cartilage represents an end-stage of OA, the result of degeneration and repair that has taken place over many years. For the treatment of OA in clinical practice, it is more relevant to evaluate more early stages of cartilage degeneration. Moreover, in addition to knowledge on proteoglycan turnover (synthesis and release) it is also important whether this beneficial effect on turnover results in actual improvement of matrix integrity as indicated by an increase in proteoglycan content that is significantly diminished in the case of OA.

For this reason, the present study evaluates the *in vitro* effect of celecoxib on human articular cartilage. Effects on degenerated (pre-clinical) OA cartilage were compared to those on established (end stage) OA and healthy cartilage.

Material and methods

CARTILAGE CULTURE TECHNIQUE

Normal healthy cartilage based on a smooth glossy appearance was obtained *post mortem* from patients without history of joint disorders (mean age 69 $\pm$ 5 years ($\pm$S.E.M.), two males, four females). Degenerated articular cartilage tissue that was obtained *post mortem* as well (mean age 80 $\pm$ 3 years ($\pm$S.E.M.), two males, two females). Degenerated cartilage was identified macroscopically on the bases of a fibrillated surface as previously reported[20]. Also, these donors had no clinical history on joint disorders. This stresses the difference between degenerative changes in cartilage that could have been visualized by radiography and actual clinical manifestations of the disease which may result in the diagnosis of OA. In general, all changes observed in degenerated cartilage are, although less pronounced, observed in osteoarthritic (OA) cartilage and are significantly different from normal healthy cartilage. For that reason degenerated cartilage obtained *post mortem* can be considered as a pre-clinical phase of OA[20]. OA cartilage obtained from patients at knee replacement surgery with diagnosed OA (mean age 69 $\pm$ 2 years ($\pm$S.E.M.), one male, five females) was obtained postoperatively. NSAID medication is stopped 7 days before surgery, thus no interference of previous medication use is to be expected. It should be kept in mind that only the cartilage that could be cut from the deteriorated joints (after replacement surgery) was used. Cartilage that appeared with full thickness with significant fibrillation was selected. Thus in fact the entire joint had a worse appearance than represented by the cartilage used for evaluation. Mostly significant parts of the joint were only covered with thin or sometimes no cartilage. OA cartilage obtained at joint replacement surgery represents an end stage of OA that reflects degenerated as well as reparative processes and in that respect differs from degenerated cartilage although the histological classification may be comparable. Cartilage obtained *post mortem* was taken within 24 h of death from human knee condyles, cartilage obtained post-surgery always within 4 h after dissection. Collection of cartilage was according to the medical ethical regulations of the University Medical Center Utrecht. Slices of cartilage were cut aseptically as thick as possible from the articular surface, excluding the underlying bone, and kept in phosphate buffered saline (PBS). Within 1 h of dissection the slices were cut into square pieces, weighed aseptically (range 5–15 mg, accuracy $\pm$0.1 mg) and cultured individually in 96-well round-bottomed microtiter plates (200 μl culture medium, 5% CO_2 in air, 37°C). The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM), supplemented with glutamine (2 mM), penicillin (100 IU/ml), streptomycin sulfate (100 μg/ml), ascorbic acid (0,085 mM), and 10% heat inactivated pooled human male AB^+ serum. Cartilage was always pre-cultured for 24 h (wash-out period), after which culture medium was refreshed before start of the experiment. In addition, three tissue samples of each donor were fixed in 4% phosphate buffered formalin for standard light microscopy. Sections were stained with safranin-O fast green-iron hematoxylin and graded for features of OA according to the slightly modified criteria[21] described by Mankin. The tidemark between cartilage and bone was not present in our cartilage samples since bone was not included. Also as a result of our dissection method, cartilage samples were not covered with pannus. Therefore, the maximum score that could be obtained was 11 instead of the original 14, when all criteria described by Mankin *et al.*[22] (including pannus, clefts to calcified zone, and tidemark crossed by blood vessels) could have been included.

EXPERIMENTAL SETUP

Healthy, early and late OA human articular cartilage tissues were cultured for 7 days. Celecoxib (supplied by Pfizer USA) was added at the start of the culture in concentrations of 0.1, 1 and 10 μM. The mean pharmacological plasma concentration was 5 μM[23]. After 4 days medium was refreshed and cartilage cultured for a successive 3 days with the same additions. Changes in cartilage matrix turnover (proteoglycan synthesis, retention, and release) and matrix integrity (proteoglycan content) were determined. Experiments were repeated at least 4 times; for each experiment, cartilage of another donor was used.

PROTEOGLYCAN ANALYSES

Sulphate incorporation rate was determined, as a measure of the proteoglycan synthesis rate, during the last 4 h of the first 4-day culture period, as described previously[24]. Before addition of $^{35}SO_4^{2-}$ ($Na_2^{35}SO_4$, 14.8 kBq/200 μl, DuPont NEX-041-H, carrier-free), culture medium was replaced by equilibrated (CO_2 and temperature) fresh medium. After 4 h labeling, the cartilage explants were rinsed 3 times for 45 min in culture medium under culture conditions and incubated for the additional period of 3 days. After this second culture period medium was removed and stored at −20°C for further analysis. Cartilage tissue samples were digested (2 h, 65°C) in papain buffer as

described before[25]. Papain digests were diluted to the appropriate concentrations and glycosaminoglycans (GAGs) were stained and precipitated with Alcian Blue dye solution[26]. The pellet obtained after centrifugation (9000*g*, 10 min) was washed once (NaAc-buffer containing 0.1 M $MgCl_2$) and subsequently dissolved in sodium dodecyl sulfate (SDS). The $^{35}SO_4^{2-}$ radioactivity of the samples was measured by liquid scintillation analysis. $^{35}SO_4^{2-}$ incorporation was normalized to the specific activity of the medium, labeling time and wet weight of the cartilage samples, and proteoglycan synthesis rate expressed as nanomoles sulfate incorporated per hour per gram wet weight of the cartilage (nmol/h/g).

Release of newly formed proteoglycans as a measure of retention of these proteoglycans was determined similarly. GAGs were precipitated from the medium obtained from day 4 to 7 and stained with Alcian Blue dye solution[27]. The radio-labeled GAGs were measured by liquid scintillation analysis and normalized to the proteoglycan synthesis rate and expressed as percentage release of newly formed proteoglycans.

For the total release of proteoglycans, the GAG in the medium obtained from days 4 to 7 were precipitated and stained with Alcian Blue as described above[24]. The GAG content in the papain digest of cartilage samples, as a measure of proteoglycan content, was analyzed the same way. Blue staining was quantified photometrically by the change in absorbance at 620 nm. Chondroitin sulfate (Sigma C4383) was used as a reference. Values for content were normalized to the wet weight of the cartilage and expressed as milligram of GAG/gram wet weight of cartilage (mg/g). Values for release were normalized to the GAG content of the explants and expressed as percentage release of GAGs.

PGE_2 AND NITRIC OXIDE (NO) DETERMINATION

PGE_2 was determined in culture medium at day 4 by Enzyme Immuno Assay (EIA, Caymann Chemical) and expressed in pg/ml. Nitrite and nitrate concentration (as a measure for NO) in the same culture medium of the first 4 days of culture was quantified by colorimetric assay based on the Griess reaction and expressed in micromolar.

CALCULATIONS AND STATISTICAL ANALYSIS

Because of focal differences in composition and bioactivity of the cartilage on the femur condyles, the results of 10 cartilage samples per parameter per donor, obtained randomly and handled individually, were averaged and taken as a representative value for the cartilage of that donor. Several experiments with each cartilage of a different donor (at least $n = 4$) were performed. Statistical evaluation of differences between healthy, degenerated and OA cartilage was performed with a non-parametric test for unpaired data (Mann–Whitney). Statistical evaluation of the effects of treatment was performed with a non-parametric test for paired data (Wilcoxon). *P* values less than or equal to 0.05 were considered statistically significant.

Results

THE EFFECT OF CELECOXIB ON NORMAL CARTILAGE

Average Mankin grade of the normal cartilage, as expected, was low; 1 ± 0.1 on a scale of 11. The cartilage had always an intact articular surface, safranin-O staining was equally distributed throughout the tissue and chondrocyte localization showed a normal appearance [Fig. 1(A) and Table I].

Celecoxib, at a concentration of 10 μM, did not change proteoglycan turnover of normal healthy human articular cartilage [dotted lines in Fig. 2(A–D)]; proteoglycan synthesis rate [Fig. 2(A)], percentage release of newly formed proteoglycans [Fig. 2(B)], as a measure of retention of these newly formed proteoglycans, and the percentage release of proteoglycans [resident ones plus newly formed, Fig. 2(C)] did not change. Also GAG content [Fig. 2(D)] was not influenced significantly by celecoxib. When release of newly formed and total amount of proteoglycans were calculated as absolute values, not normalized to synthesis rate and content, respectively, also no effects of celecoxib on normal cartilage were found (data not shown). Also at a concentration of 0.1 and 1 μM no effects were found as reported previously[18].

EFFECTS OF CELECOXIB ON DEGENERATED (PRE-CLINICAL OA) CARTILAGE

Mostly treatment with NSAIDs will take place on early stages of joint degeneration. Therefore, we tested celecoxib on degenerated (pre-clinical OA) cartilage. The degenerated cartilage had on average a modified Mankin score of 4 ± 0.5 (Table I). In the degenerated tissue, surface deterioration was visible. The safranin-O staining was lost from the surface layer of the degenerated samples and chondrocyte distribution was disturbed [Fig. 1(B)]. The early OA cartilage showed the typical intermediate biochemical features with respect to proteoglycan turnover when compared to healthy human cartilage and OA cartilage (Table I)[20].

When degenerated cartilage was incubated with celecoxib, in a concentration of 0.1–10 μM, a slight but dose-dependent increase of the cartilage matrix synthesis was observed [Fig. 2(A) squares] at all concentrations tested statistically significant compared to untreated control degenerated cartilage. The actual synthesis rate reached even higher levels than normal cartilage although not statistically significant.

Release of newly formed proteoglycans, as absolute values (data not shown) and as percentage (normalized to the proteoglycan synthesis rate) was significantly reduced up to nearly complete normalization by the addition of celecoxib [at all concentrations tested statistically significant; Fig. 2(B) squares]. Although there appeared to be a slight dose dependency, statistically, normalization was reached already at the lowest concentration tested.

Similar results were found for the release of all proteoglycans, newly formed plus resident proteoglycans, when calculated as absolute (data not shown) and normalized to the proteoglycan content representing percentage total proteoglycan release [Fig. 2(C) squares]. Again we found a reduction up to complete normalization under the influence of celecoxib. Already at the lowest concentration used, the addition of celecoxib resulted in a retention of newly formed proteoglycans not different from normal healthy cartilage anymore.

With respect to matrix integrity celecoxib was able to induce normalization in proteoglycan content [Fig. 2(D) squares] that was statistically significant at all concentrations tested.

EFFECTS OF CELECOXIB ON OA CARTILAGE

The OA cartilage had on average a modified Mankin score of 5 ± 0.5, slightly higher than that of degenerative

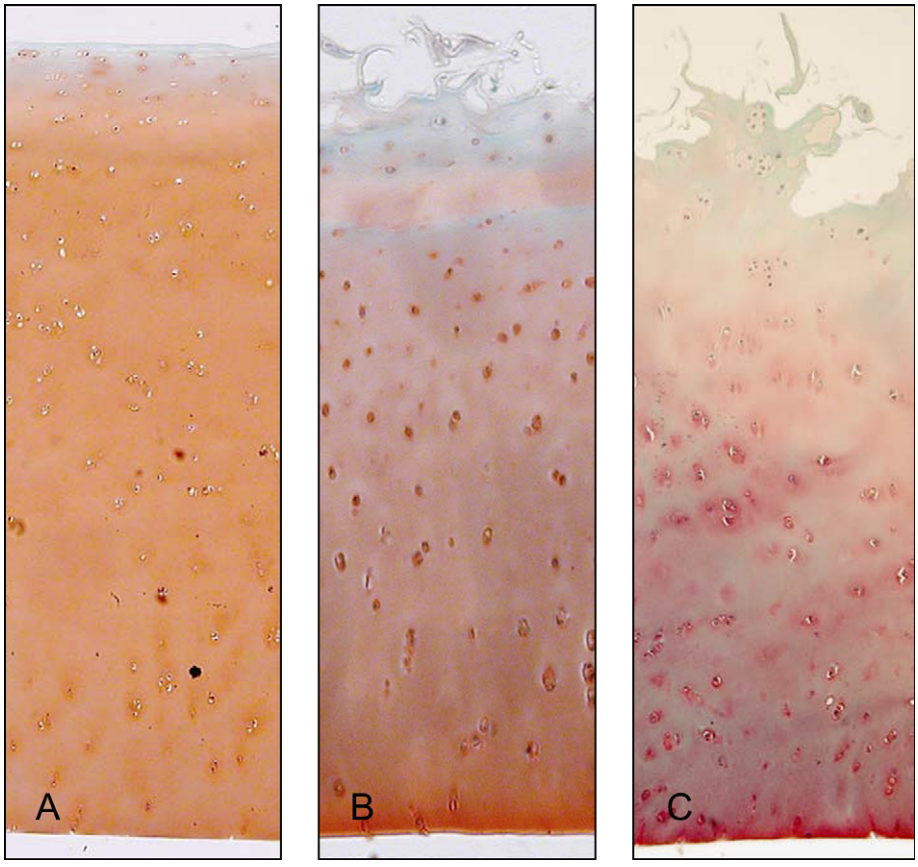

Fig. 1. Normal healthy, degenerative and osteoarthritic cartilage histology. Representive light micrographs of condylar cartilage obtained *post mortem* from joints with normal healthy cartilage (A), or joints with degenerated cartilage (B) and cartilage obtained at joint replacement surgery (C). Sections are stained with safranin-O fast green-iron hematoxylin and graded for features of OA according to the slightly modified criteria described by Mankin *et al.*, scores for the depicted samples are 0, 4 and 6, respectively.

cartilage (Table I) and significantly higher than that of normal cartilage. It should be kept in mind that only the cartilage that could be cut from the joint surfaces after replacement surgery was used. Thus in fact the entire joint had a worse appearance than represented by the modified Mankin score of the cartilage used. The OA cartilage surface deterioration was clearly visible by light microscopy. The safranin-O staining was lost from the surface layer of the degenerated samples and chondrocyte distribution was disturbed [clusters of chondrocytes in the surface layer of the cartilage were visible; Fig. 1(C)]. The OA cartilage showed the typical biochemical features with respect to proteoglycan turnover when compared to healthy human cartilage (Table I) and these changes always exceeded the

Table I
The histological and biochemical characteristics of human healthy cartilage, degenerative and osteoarthritic cartilage

	Normal cartilage (average ± S.E.M.; $n = 4$)	Degenerative cartilage (average ± S.E.M.; $n = 4$)	$P<$	OA cartilage (average ± S.E.M.; $n = 6$)	$P<$
Histological cartilage damage	1 ± 0.1	4 ± 0.5	0.05	5 ± 0.5	0.05
PG synthesis (nmol/h/g)	10 ± 1	8 ± 3	ns	5 ± 2	0.05
% New release (%)	6 ± 1	8 ± 1	0.01	11 ± 1	0.01
% Total release (%)	3 ± 1	5 ± 1	0.03	6 ± 1	0.02
PG-content (mg/g)	35 ± 2	23 ± 5	0.03	18 ± 3	0.01
Prostaglandin E_2 (pg/ml)	64 ± 9	129 ± 12	0.03	215 ± 34	0.03
Nitric oxide (μM)	2.9 ± 0.3	4.3 ± 1.1	ns	8.0 ± 1.6	0.05

Proteoglycan synthesis rate as a measure of cartilage matrix synthesis, percentage release of newly formed proteoglycans as a measure of retention for the newly formed proteoglycans (normalized to the synthesis of these proteoglycans), percentage total release of proteoglycans, calculated by the percentage release of GAG (normalized to the GAG content), proteoglycan content, PGE_2 and NO. The results are presented as means of at least four experiments (viz. four cartilage donors) ±S.E.M.. Statistical evaluation of differences between healthy human articular cartilage and degenerated or OA cartilage was performed with a non-parametric test for unpaired data. *P* values less than or equal to 0.05 were considered statistically significant.

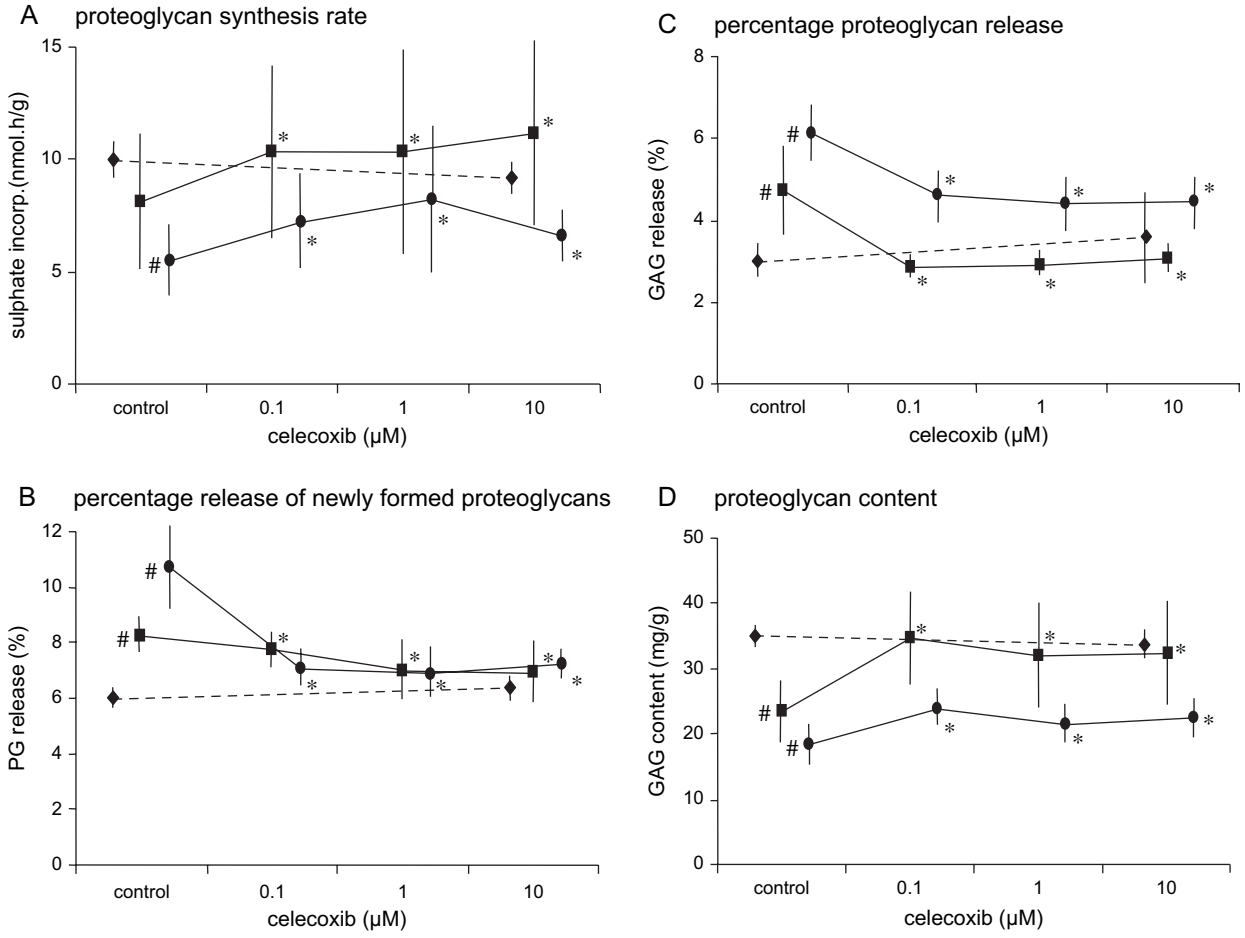

Fig. 2. The effect of celecoxib on degenerated and OA human articular cartilage explants. Proteoglycan synthesis rate as measure of cartilage matrix synthesis (A), percentage release of newly formed proteoglycans (B) as a measure of retention for the newly formed proteoglycans (normalized to the synthesis of these proteoglycans), percentage total release of proteoglycans (C), measured by the percentage release of GAG (normalized to the GAG content), and proteoglycan content (D) are depicted. Diamonds represent effects of celecoxib (only 10 μM) on normal cartilage, squares represent effects of celecoxib on degenerated cartilage, and dots represent effects of celecoxib on OA cartilage. The results are presented as means of at least four experiments with each cartilage of a different donor ±S.E.M. Statistically differences between degenerated and OA cartilage compared to normal cartilage, calculated by non-parametric unpaired analysis ($P < 0.05$) at baseline are marked with an #. Statistically differences of the effects of celecoxib compared to controls, calculated by non-parametric paired analysis ($P < 0.05$) are marked with an asterisk.

changes as observed in degenerated cartilage. The effects of celecoxib, observed for late-stage OA cartilage, were similar as for degenerative OA cartilage [Fig. 2(A–D)].

For all proteoglycan parameters there was a statistically significant improvement found when celecoxib was added. However, both proteoglycan synthesis rate and proteoglycan content remained statistically significant below healthy cartilage control values. None of the effects of celecoxib on OA cartilage were statistically significantly different from the effects on degenerated cartilage.

EFFECT OF CELECOXIB ON PGE_2 AND NO PRODUCTION

In addition to proteoglycan turnover and content, we have determined the effect of celecoxib on PGE_2 production as a indirect measurement of COX-2 activity. To demonstrate that the effects of celecoxib were COX-2 (viz. PGE_2) related we also quantified the NO production of the different types of cartilage.

In both degenerated and OA cartilage the amount of PGE_2 formed and measured in the supernatant of the cartilage cultures was significantly elevated compared to normal cartilage (Table I). OA cartilage showed the highest amounts of PGE_2, most likely due to the strongest COX-2 activity, where the basal level of normal cartilage is most likely due to non or low COX-2 activity.

Similar results were found for the NO levels (Table I). In both types of diseased cartilage NO was elevated (on average 2.9 ± 0.3 μM, 4.3 ± 1.1 μM, and 8.0 ± 1.6 μM for normal, degenerated, and clinical OA cartilage, respectively), again with the highest levels found in the OA cartilage.

The basal PGE_2 production of normal cartilage was not influenced by celecoxib at a concentration of 10 μM (Fig. 3, open bars). In contrast, when degenerated cartilage was incubated with celecoxib, normalization of the elevated PGE_2 levels occurred in a dose-dependent manner (Fig. 3 gray bars). Also the elevated PGE_2 levels of OA cartilage could be normalized, in a dose-dependent manner, when incubated with celecoxib (Fig. 3, black bars). The normalization of PGE_2 production in both degenerated and OA cartilage shows indirectly that celecoxib inhibits COX-2 activity in degenerative and OA cartilage.

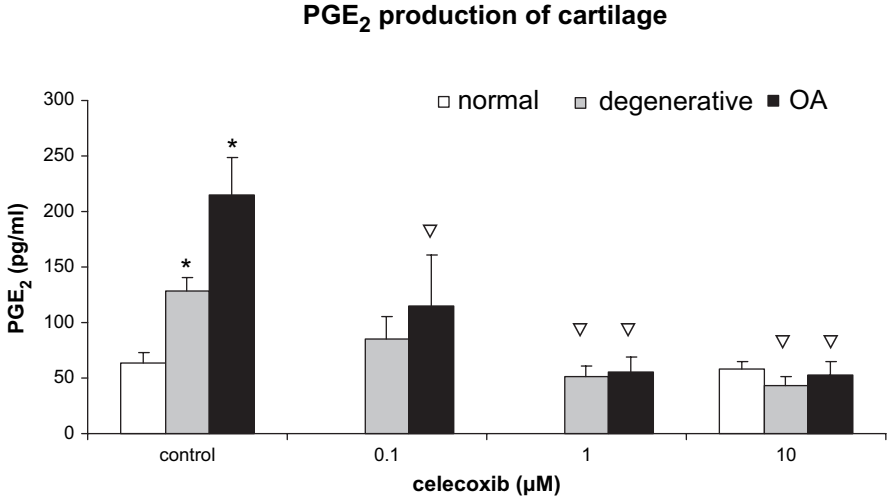

Fig. 3. The effect of celecoxib on PGE_2 of normal, degenerated and OA human articular cartilage explants. PGE_2 production of normal, degenerated, and OA cartilage with or without celecoxib are depicted. Open bars represent effects of celecoxib on normal cartilage, gray bars represent effects of celecoxib on degenerated cartilage, and black bars represent effects of celecoxib on OA cartilage. The results are presented as means of at least four experiments with each cartilage of a different donor $\pm$S.E.M. Statistically differences between degenerated and OA cartilage compared to normal cartilage, calculated by non-parametric unpaired analysis ($P < 0.05$) at baseline are marked with an asterisk. Statistically differences of the effects of celecoxib compared to untreated controls, calculated by non-parametric paired analysis ($P < 0.05$) are marked with a triangle.

In contrast to PGE_2, the NO production of normal, degenerated and OA cartilage was not influenced by celecoxib (on average $3.1 \pm 0.9\,\mu M$, $4.3 \pm 2.5\,\mu M$ and $7.0 \pm 1.5\,\mu M$ for normal, degenerated and clinical OA cartilage incubated with $10\,\mu M$ celecoxib, respectively).

Discussion

Our study shows that the selective COX-2 inhibitor celecoxib has a favorable effect on proteoglycan synthesis, retention, release and content of both degenerated (pre-clinical) and (late-stage) OA cartilage. On normal healthy cartilage no effects were observed, confirming our previous results[18] where we showed in a complete dose response curve that normal cartilage remained unaffected. Our results with respect to proteoglycan synthesis and retention of late-stage OA cartilage corroborate recent findings by El Hajjaji *et al.*[19] using a slightly different approach. In their study, they also demonstrated a beneficial effect on hyaluronan synthesis in human late-stage OA cartilage obtained at joint replacement surgery. The present study adds in this respect that release of resident proteoglycans also and more importantly, proteoglycan content is beneficially influenced by celecoxib. In addition, our study shows that also for degenerated cartilage a chondroprotective effect of celecoxib can be observed.

All beneficial effects on cartilage proteoglycan turnover and content were similar for OA cartilage obtained at joint replacement surgery and degenerated (pre-clinical) cartilage. However, especially degenerated cartilage seems to profit from COX-2 inhibition as all four parameters normalized whereas for OA cartilage COX-2 inhibition normalized only retention and release. This, and the fact that normal healthy cartilage stays unaffected is of major importance in the treatment of patients with OA. This could mean that treatment of OA patients with celecoxib specifically at an early stage of their disease could slowdown further damage and with that postpone further invasive chirurgical treatment (joint replacement surgery). These effects contrast the results of several other studies obtained with NSAIDs tested under comparable conditions. Frequently used NSAIDs such as naproxen and ibuprofen have been demonstrated to inhibit the synthesis of cartilage proteoglycans[4,5,8,27] and to increase the release of proteoglycans[27]. However, although mostly effects have been reported to be adverse, also no or positive effects of NSAIDs have been described[4,5,8,27,28].

These are all direct effects on cartilage and should be seen in the context of the significant anti-inflammatory effects of these NSAIDs. By inhibiting joint inflammation they may indirectly be beneficial to cartilage, specifically when inflammation is primary in the cause of cartilage damage as, e.g., in rheumatoid arthritis. In the case of OA, where inflammation may contribute but is not primarily responsible for cartilage damage, adverse direct effects of NSAIDs on cartilage during long-term treatment may have important impact on long-term outcome.

This study describes a beneficial effect of a selective COX-2 inhibitor on OA cartilage proteoglycan turnover and content, whereas no effects are observed on normal healthy cartilage. This could be due to better accessibility of celecoxib in (pre-clinical) OA cartilage, due to its fibrillated surface. However, in a previous study[18] we showed that healthy cartilage with an intact surface, when co-cultured with peripheral blood mononuclear cells of rheumatoid arthritis patients or with a combination of IL-1β and TNFα demonstrated the same cellular characteristics, as OA chondrocytes in the present study, which could be normalized by the addition of celecoxib. This suggests that the difference between normal and (pre-clinical) OA cartilage is not due to accessibility. Furthermore, the elevated PGE_2 levels in degenerative and even more elevated in OA cartilage (according to literature[15,16]), that could be normalized by addition of celecoxib suggest COX-2 regulation to be involved, rather than just accessibility. Another important mediator of cartilage damage is NO. The production of NO in joint pathology could contribute to

disease pathogenesis in variety of ways[29]. These effects of NO on synovial cells and chondrocytes include: inhibition of collagen and proteoglycan synthesis; activation of metalloproteinases; and apoptosis[30]. It appeared that celecoxib was not able to influence the elevated NO production of degenerative and OA cartilage in our study. This implies that the favorable effect of celecoxib on (pre-clinical) OA cartilage is mainly due to inhibiting COX-2 activity. The remaining elevated levels of NO could explain why COX-2 inhibition does not normalize for both types of diseased cartilage all proteoglycan parameters. Especially proteoglycan synthesis seems to favor only partially from COX-2 inhibition. This fits with the knowledge that high levels of NO decrease PG synthesis[30].

The presence of COX-2 in OA cartilage, directly measured[15,16] or indirectly via PGE_2[17], is evident. It could be, although speculative, that the adverse affects of some of the conventional NSAIDs on the cartilage results from inhibition of COX-1, which could be essential for normal chondrocyte function. However, other effects of these NSAIDs, COX independent, might be involved as well[9]. The other way around, whether selective COX-2 inhibitors, other than celecoxib, are as beneficial for (pre-clinical) OA cartilage remains to be established as well. All the effects found in the present study are beneficial to cartilage. Moreover, the response is obtained with a clinical relevant concentration of the drug because the mean pharmacological plasma concentration is 5 μM[23]. The response is also quick, within 7 days chondrocytes appear to be able to start restoration of the extracellular matrix. Whether these promising effects also take place *in vivo* remains of course to be seen. *In vivo* animal studies and clinical trials have to be performed to prove the suggestive chondroprotection of COX-2 inhibition found *in vitro*. The present results imply an important role for COX-2 in the disturbed proteoglycan turnover in OA, making COX-2 inhibitors a relevant choice of treatment in the elderly. On the other hand, questions have been raised about cardiovascular side effects, and about possible adverse effects on bone healing. These effects appear to be shared with non-selective NSAIDs[31–33].

In conclusion, although *in vitro* findings, the present study suggests that besides the anti-inflammatory and analgesic characteristics of selective COX-2 inhibitors, celecoxib has cartilage protective capacities, contributing directly to improvement of cartilage matrix integrity in OA already in an early phase, which expectedly depends on its selective COX-2 inhibition.

References

1. Aspden RM, Scheven BA, Hutchison JD. Osteoarthritis as a systemic disorder including stromal cell differentiation and lipid metabolism. Lancet 2001;357: 1118–20.
2. Creamer P, Hochberg MC. Osteoarthritis. Lancet 1997; 350:503–8.
3. Poole AR. An introduction to the pathophysiology of osteoarthritis. Front Biosci 1999;4:D662–70.
4. Collier S, Ghosh P. Comparison of the effects of non-steroidal anti-inflammatory drugs (NSAIDs) on proteoglycan synthesis by articular cartilage explant and chondrocyte monolayer cultures. Biochem Pharmacol 1991;41:1375–84.
5. Ding C. Do NSAIDs affect the progression of osteoarthritis? Inflammation 2002;26:139–42.
6. Henrotin YE, Labasse AH, Simonis PE, Zheng SX, Deby GP, Famaey JP, *et al*. Effects of nimesulide and sodium diclofenac on interleukin-6, interleukin-8, proteoglycans and prostaglandin E2 production by human articular chondrocytes *in vitro*. Clin Exp Rheumatol 1999;17:151–60.
7. Johnston SA, Fox SM. Mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis. J Am Vet Med Assoc 1997;210: 1486–92.
8. Smith RL, Kajiyama G, Lane NE. Nonsteroidal antiinflammatory drugs: effects on normal and interleukin 1 treated human articular chondrocyte metabolism *in vitro*. J Rheumatol 1995;22:1130–7.
9. Abramson S, Weissmann G. The mechanisms of action of nonsteroidal antiinflammatory drugs. Clin Exp Rheumatol 1989;7(Suppl 3):S163–70.
10. Brideau C, Kargman S, Liu S, Dallob AL, Ehrich EW, Rodger IW, *et al*. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. Inflamm Res 1996;45:68–74.
11. DuBois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, van de Putte LB, *et al*. Cyclooxygenase in biology and disease. FASEB J 1998;12:1063–73.
12. Cryer B, Dubois A. The advent of highly selective inhibitors of cyclooxygenase—a review. Prostaglandins Other Lipid Mediat 1998;56:341–61.
13. Fung HB, Kirschenbaum HL. Selective cyclooxygenase-2 inhibitors for the treatment of arthritis. Clin Ther 1999;21:1131–57.
14. Hawkey CJ. COX-2 inhibitors. Lancet 1999;353: 307–14.
15. Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, *et al*. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum 2002;46:1789–803.
16. Siegle I, Klein T, Backman JT, Saal JG, Nusing RM, Fritz P. Expression of cyclooxygenase 1 and cyclooxygenase 2 in human synovial tissue: differential elevation of cyclooxygenase 2 in inflammatory joint diseases. Arthritis Rheum 1998;41:122–9.
17. Jacques C, Sautet A, Moldovan M, Thomas B, Humbert L, Berenbaum F. Cyclooxygenase activity in chondrocytes from osteoarthritic and healthy cartilage. Rev Rhum Engl Ed 1999;66:701–4.
18. Mastbergen SC, Lafeber FP, Bijlsma JW. Selective COX-2 inhibition prevents proinflammatory cytokine-induced cartilage damage. Rheumatology (Oxford) 2002;41:801–8.
19. El Hajjaji H, Marcelis A, Devogelaer JP, Manicourt DH. Celecoxib has a positive effect on the overall metabolism of hyaluronan and proteoglycans in human osteoarthritic cartilage. J Rheumatol 2003;30: 2444–51.
20. van Valburg AA, Wenting MJ, Beekman B, Te KJ, Lafeber FP, Bijlsma JW. Degenerated human articular cartilage at autopsy represents preclinical osteoarthritic cartilage: comparison with clinically defined osteoarthritic cartilage. J Rheumatol 1997;24:358–64.
21. Lafeber FP, van der Kraan PM, van Roy HL, Vitters EL, Huber-Bruning O, van den Berg WB, *et al*. Local changes in proteoglycan synthesis during culture are different for normal and osteoarthritic cartilage. Am J Pathol 1992;140:1421–9.
22. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular

cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523–37.
23. Paulson SK, Hribar JD, Liu NW, Hajdu E, Bible RH Jr, Piergies A, *et al*. Metabolism and excretion of [(14)C]celecoxib in healthy male volunteers. Drug Metab Dispos 2000;28:308–14.
24. Lafeber FP, Vander Kraan PM, Van Roy JL, Huber-Bruning O, Bijlsma JW. Articular cartilage explant culture; an appropriate *in vitro* system to compare osteoarthritic and normal human cartilage. Connect Tissue Res 1993;29:287–99.
25. Lafeber FP, Vander Kraan PM, Huber-Bruning O, Vanden Berg WB, Bijlsma JW. Osteoarthritic human cartilage is more sensitive to transforming growth factor beta than is normal cartilage. Br J Rheumatol 1993;32:281–6.
26. Whiteman P. The quantitative determination of glycosaminoglycans in urine with Alcian Blue 8GX. Biochem J 1973;131:351–7.
27. Dingle JT. The effect of nonsteroidal antiinflammatory drugs on human articular cartilage glycosaminoglycan synthesis. Osteoarthritis Cartilage 1999;7:313–4.
28. Dingle JT. The effects of NSAID on the matrix of human articular cartilages. Z Rheumatol 1999;58:125–9.
29. Abramson SB, Amin AR, Clancy RM, Attur M. The role of nitric oxide in tissue destruction. Best Pract Res Clin Rheumatol 2001;15:831–45.
30. Amin AR, Mandar D, Attur M, Abramson SB. COX-2, NO, and cartilage damage and repair. Curr Rheumatol Rep 2000;2:447–53.
31. Harris RC Jr. Cyclooxygenase-2 inhibition and renal physiology. Am J Cardiol 2002;89:10D–7D.
32. Ray WA, Stein CM, Daugherty JR, Hall K, Arbogast PG, Griffin MR. COX-2 selective non-steroidal anti-inflammatory drugs and risk of serious coronary heart disease. Lancet 2002;360:1071–3.
33. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. J Bone Miner Res 2002;17:963–76.