Research article

Cyclooxygenase inhibition lowers prostaglandin E\(_2\) release from articular cartilage and reduces apoptosis but not proteoglycan degradation following an impact load \textit{in vitro}

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\textbf{Abstract}

This study investigated the release of prostaglandin E\(_2\) (PGE\(_2\)) from cartilage following an impact load \textit{in vitro} and the possible chondroprotective effect of cyclooxygenase-2 (COX-2) inhibition using non-steroidal anti-inflammatory drugs (NSAIDs).

Explants of human articular cartilage were subjected to a single impact load in a drop tower, and then cultured for 6 days in the presence of either a selective COX-2 inhibitor (celecoxib; 0.01, 0.1, 1.0 and 10 \(\mu\)M) or a non-selective COX inhibitor (indomethacin; 0.1 and 10 \(\mu\)M). The concentrations of PGE\(_2\) and glycosaminoglycans (GAGs), a measure of cartilage breakdown, were measured in the explant culture medium at 3 and 6 days post-impact. Apoptotic cell death was measured in frozen explant sections by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) method.

PGE\(_2\) levels were increased by more than 20-fold in the medium of explants at both 3 (\(p = 0.012\)) and 6 days (\(p = 0.004\)) following impact, compared with unloaded controls. In the presence of celecoxib and indomethacin, the PGE\(_2\) levels were reduced in a dose-related manner. These inhibitors, however, had no effect in reducing the impact-induced release of GAGs from the cartilage matrix. Addition of celecoxib and indomethacin significantly reduced the number of trauma-induced apoptotic chondrocytes in cartilage explant sections.

In this study, a marked increase in PGE\(_2\) was measured in the medium following an impact load on articular cartilage, which was abolished by the selective COX-2 inhibitor, celecoxib, and non-selective indomethacin. These inhibitors reduced chondrocyte apoptosis but no change was observed in the release of GAGs from the explants, suggesting that the COX/PGE\(_2\) pathway is not directly responsible for cartilage breakdown following traumatic injury. Our \textit{in vitro} study demonstrates that it is unlikely that COX-2 inhibition alone would slow down or prevent the development of secondary osteoarthritis.

\textbf{Introduction}

Articular cartilage is a highly specialised connective tissue that covers the ends of long bones in diarthrodial joints. The tissue protects the joint by distributing applied loads and providing a low-friction, wear-resistant, lubricated surface to facilitate movement. The cartilage matrix consists of collagen fibres that reinforce a proteoglycan gel. The main proteoglycan is aggrecan, which comprises a protein core highly substituted with polysulfated glycosaminoglycan (GAG) side chains.

Traumatic joint damage, such as may be sustained in a road traffic accident or a sporting injury, is a known risk factor for the subsequent development of secondary osteoarthritis (OA) [1]. Injury can result in progressive cartilage loss causing pain, swelling, inflammation and joint immobility. Ultimately, a joint replacement may be required. However the processes resulting in cartilage breakdown following injury and the ability of the tissue to repair itself are poorly understood. In humans, studies have shown elevated levels of breakdown products from cartilage matrix many years after injury [2-4]. The relationship between joint injury and OA development has also been

COX = cyclooxygenase; ELISA = enzyme-linked immunosorbent assay; GAG = glycosaminoglycan; IL = interleukin; MMP = matrix metalloproteinase; iNOS = inducible nitric oxide synthase; NSAID = non-steroidal anti-inflammatory drug; OA = osteoarthritis; PGE\(_2\) = prostaglandin E\(_2\); RA = rheumatoid arthritis; TNF\(\alpha\) = tumour necrosis factor \(\alpha\).
demonstrated in various animal models both in vivo and in vitro [5,6].

Under normal physiological loading, articular cartilage is subjected to a variety of stresses. These biomechanical factors are believed to stimulate chondrocyte metabolism, providing a mechanism for the cartilage to adapt to the demands imposed by the body. However, in abnormal or injurious joint loading the balance between cartilage matrix synthesis and degradation is disturbed [7], resulting in tissue breakdown and the risk of progression of OA.

There is considerable evidence that the cytokine interleukin-1 (IL-1) plays an important role in OA, being up-regulated in OA synovium and cartilage [8,9]. IL-1β expression in articular cartilage is also regulated by mechanical factors [10]. It induces a catabolic cascade involving the cyclooxygenase (COX) enzymes; two isoforms of which, COX-1 and COX-2, catalyse the conversion of arachidonic acid to prostaglandins (PG), the major pro-inflammatory product being prostaglandin E₂ (PGE₂) [11]. COX-1 is the constitutive form of the enzyme, naturally expressed at low levels and essential to the normal function of many tissues, whereas COX-2 is the inducible form, which is commonly up-regulated following an insult to the tissue [12]. Consequently, PGE₂ has been found to be elevated in cartilage, synovium and synovial fluid in OA joints [13,14] and also in normal cartilage by prolonged static mechanical loads [15]. Similarly COX-2, but not COX-1, has been shown to be up-regulated in chondrocytes of OA cartilage [16]. This COX-2/PGE₂ pathway is of major interest in OA as the first line of treatment in this disease is the use of the non-steroidal anti-inflammatory drugs (NSAIDs) for pain relief. These drugs inhibit the activity of COX [17]. The non-selective NSAIDs inhibit both COX-1 and COX-2 (e.g. indomethacin), and more recently NSAIDs have been developed that are more selective for COX-2 (e.g. celecoxib) exhibiting fewer unwanted side effects.

Several studies, both in animal models [18] and in human joints [19,20], have shown that apoptosis (programmed cell death) is an important factor in the progression of OA. A positive correlation exists between severity of OA and percentage of apoptotic cells [21]. Apoptosis occurs following mechanical injury [22-26] and is thought to be initiated by IL-1β (in the presence of tumour necrosis factor (TNF) α; [27]), which in turn activates the caspase cascade [28]. As IL-1β is also involved in activating the COX/PGE₂ pathway and PGE₂ is reported to induce apoptosis in bovine articular chondrocytes [29], the prostanoïd may have a role in the increased apoptosis observed following trauma.

In an attempt to understand the physical and biochemical changes occurring in articular cartilage following trauma, an in vitro impact model has been developed [7,30]. This consists of an instrumented drop tower, which enables an explant of cartilage to be subjected to a controlled impact load [31].

The aim of this study was to ascertain whether PGE₂ was released by articular cartilage chondrocytes following an impact load, and whether COX-2 inhibition using NSAIDs could provide a chondroprotective role by preventing matrix degradation. In addition, the role of COX inhibitors on trauma-induced chondrocyte apoptosis was investigated.

Materials and methods
Cartilage explants
Human articular cartilage was obtained from femoral heads retrieved during hemiarthroplasty for fractured neck of femur within 12 h of surgery. Local Ethics Committee approval was granted for this procedure. Full depth, circular explants (5 mm diameter) of articular cartilage were removed from the underlying subchondral bone of human femoral heads (n = 3; ages 58, 63 and 68 years) using a cork borer and scalpel, and cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Paisley, UK) supplemented with 10% foetal calf serum (Globepharm, Guildford, UK), 100 IU/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B (Gibco) and 25 μg/ml ascorbic acid (Sigma-Aldrich Co. Ltd., Gillingham, UK). To minimise the effect of site variation and differing cartilage thicknesses, test and control explants were taken from adjacent sites over the femoral head. In this study, cartilage was removed from the underlying bone as the subchondral bone is often too fragile and the surface too uneven for impact loading. Following removal, and prior to impact loading, each explant was placed in 2 ml of culture medium in a 24-well plate and placed in an incubator at 37°C with 5% CO₂. The explants were allowed to equilibrate for 72 h as Fémor et al. showed that there was an initial increase in PGE₂ release from harvested cartilage explants, and that this stabilised after 72 h in culture and remained stable for up to 7 days [32]. The wet weight of each explant was measured prior to loading by placing in a sterile pre-weighed microcentrifuge tube containing DMEM.

Impact loading
A specially designed drop tower was used to drop a mass on to a cartilage explant from a known height [7,30,31]. The explants were placed individually on the loading platen and subjected to a single impact load using a 500 g mass dropped from a height of 25 mm. The duration of each impact was approximately 3 ms, with an energy of 0.13 J and a peak stress of around 25 MPa. Impact loading conditions were chosen to produce moderate, but not overly severe, damage to the tissue based on our previous studies [7,30,33]. Control explants were placed in the machine but not loaded. Following impact, both control and loaded explants were re-cultured in fresh medium (1 ml per explant) for 6 days in the presence of either the selective COX-2 inhibitor celecoxib (donated by Pfizer Inc., New York, USA; 0.01, 0.1, 1.0 and 10 μM) or indomethacin
(Sigma; 0.1 and 10 μM), which is non-selective and inhibits both COX-1 and COX-2. The experiment was performed three times, this being enough to obtain sufficient statistical power; each femoral head yielded eight treatment groups, each containing at least five explants (i.e. unloaded controls, loaded without inhibitor, four loaded with different concentrations of celecoxib and two loaded with different concentrations of indomethacin added to the medium). Ethanol was used as the solvent for the NSAIDs with a final concentration in the medium of <0.01% v/v. Ethanol was added at the same concentration to the medium of the control explants. The ranges of inhibitor concentrations in the culture medium were chosen to include peak plasma levels of drug found in vivo. This was 1.8 μM for celecoxib following a single dose of 200 mg and 5.6–8.4 μM for indomethacin [34]. After 3 days the culture medium was collected. Fresh medium, containing inhibitors as before, was added to each culture well for a further 3 days. The medium samples collected at days 3 and 6 were stored at -20°C.

**PGE2 release assay**
PGE2 production was measured in the explant culture medium using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Prostaglandin E2 immunoassay, R&D Systems Ltd., Abingdon, UK). Results shown are measurements of total PGE2 synthesis after 3 days and after 6 days (combining the two 3-day periods).

**Glycosaminoglycan release assay**
The concentration of GAGs, a measure of cartilage breakdown, was determined in the culture medium using the 1,9-dimethylmethylene blue (DMMB) assay [35]. The method used was modified from that described by Stone et al. [36] for use in a 96-well plate. Standard curves were obtained using concentrations of chondroitin 6-sulfate (Sigma) from 0–150 μg/ml at 10 μg/ml intervals. Duplicate aliquots (10 μl) of explant culture medium and standards were mixed with 200 μl of DMMB working solution in a 96-well plate, and the absorbance read at 525 nm using a Dynatech MR5000 spectrophotometer (Dynex Technologies Ltd., Worthing, UK) plate reader 3 min after the addition of the dye. Biolinx software was used to generate a standard curve and determine the concentration of GAG in each medium sample. PGE2 and GAG concentrations were normalised to the wet weight of each explant and expressed per mg of cartilage.

**Apoptosis detection**
Articular cartilage explants were removed after 6 days in culture following impact load. They were snap frozen in OCT embedding medium (Raymond A Lamb Ltd., Eastbourne, UK) in isopentane cooled over liquid nitrogen. Four frozen (8 μm) cartilage sections were cut from each explant, collected and air dried onto SuperFrost Plus microscope slides (VWR International, Lutterworth, UK). Apoptosis was evaluated by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling) staining using an ApoAlert DNA fragmentation assay kit (BD Biosciences Clontech, Palo Alto, CA, USA) following the manufacturer’s protocol. Following TUNEL staining, the sections were stained with propidium iodide (PI, 20 μg/ml; Sigma) for 8 min at room temperature to counterstain the nuclei red. Positive control sections were treated with DNase I (1 μg/ml; Roche Diagnostics Ltd., Lewes, UK) for 10 min before TUNEL staining and negative control sections were treated with the nucleotide mix minus the TdT enzyme. Following TUNEL staining, all sections were washed and covered with Vectorshield fluorescent mounting medium (Vector Laboratories Inc., Peterborough, UK). Images of each section were taken, using the appropriate filters, with a digital camera (CoolSNAP, Roper Scientific GmbH, Germany) attached to a fluorescence microscope (Zeiss, Welwyn Garden City, UK). The percentage of apoptotic cells was determined in each of the four sections for each explant by first counting the green (fluorescein) apoptotic cells followed by the total cell count (PI, red) with the aid of image analysis software (Image J, NIH, Bethesda, MD, USA). In order to validate the TUNEL method, some sections were stained with haematoxylin and eosin and the percentage of apoptotic cells counted by observing the morphology of the nucleus and cell membrane.

**Statistical analysis**
Differences in PGE2, GAG release and percentage apoptosis between treatment groups were assessed using the unpaired Student t test (two-tailed) using SPSS v.14 software (SPSS Inc., Chicago, IL, USA). A p value less than 0.05 was considered significant. In Figures 1, 2, 3, 4, asterisks denote significant differences; *p ≤ 0.05, **p ≤ 0.01. All data are expressed as mean ± standard deviation (SD).

**Results**
PGE2 levels were increased 22-fold in the medium of explants at 3 days (p = 0.012) following impact compared to unloaded controls. By 6 days this increased further to 27-fold (p = 0.004) (Figure 1a). In the presence of celecoxib and indomethacin, the PGE2 levels were reduced in a dose-related manner both at 3 days and, more significantly, 6 days (Figure 1b). At the highest concentration (10 μM) the levels were reduced to those of the unloaded controls by both inhibitors at both timepoints. The baseline concentrations in the medium of control human cartilage explants were similar to those in other in vitro studies [37-39].

The concentration of GAGs in the medium was significantly higher in the loaded explants than in the unloaded controls at both day 3 (p = 0.010) and day 6 (p = 0.003) following impact (Figure 2a). However the addition of celecoxib or indomethacin to the culture medium had no effect on the release of GAGs from the cartilage matrix at either 3 days or 6 days (Figure 2b). Therefore, in this study, COX inhibition following impact load did not prevent proteoglycan depletion of cartilage.
An impact load increased the percentage of apoptotic cells compared with unloaded controls ($p = 0.022$). The apoptotic chondrocytes were evenly distributed throughout the zones of the loaded cartilage sections (Figure 3). Addition of the COX inhibitors to the medium reduced the percentage of impact-induced apoptosis at all doses and reached significance at 0.1 μM celecoxib ($p = 0.034$) and 10 μM indomethacin ($p = 0.032$) (Figure 4).

**Discussion**

A single impact load resulted in a marked increase in PGE$_2$ release, the number of apoptotic cells, and the concentration
of GAGs in the culture medium. Could the elevated levels of PGE₂ lead directly to these other changes and hence provide a target for early therapeutic intervention to delay or, even, prevent later secondary OA?

Tissue damage resulted in an increase in PGE₂ released to the culture medium so that by day 3 after impact, the concentration was more than 20 times higher than in control groups. The detailed course over time of this was not studied, but release continued – albeit at a slower rate – over the next 3-day period. This release could be inhibited in a dose-dependent fashion by both celecoxib and indomethacin. Apoptosis was also reduced but not in the same pattern. Indomethacin halved the number of apoptotic cells at a concentration of 1 μM and

![Figure 2](http://arthritis-research.com/content/9/6/R129)
halved it again at 10 μM. In contrast, celecoxib approximately halved the number of apoptotic cells but concentration appeared to have little effect; maximal effect was found at 0.1 μM, but this was not significantly different from any of the other concentrations used. Despite the reduction in apoptosis, neither inhibitor had any effect on matrix degradation, as indicted by there being no change in GAG release.

The release of PGE₂ by articular chondrocytes and its inhibition by indomethacin is well established [40]. Once released, however, the effects of this prostanoid on the cartilage matrix...
are rather unclear. PGE₂ is reported to have anabolic effects on cartilage: increasing proteoglycan and collagen synthesis [41], stimulating proliferation and aggrecan synthesis [42], up-regulating glucocorticoid receptors [43] and, at low concentrations, stimulating collagen II synthesis. All these effects are possibly mediated by insulin-like growth factor 1 (IGF-1) via an autocrine loop [44]. However, this response was reported to be biphasic in chondrocytes in vitro because at higher concentrations of PGE₂ collagen synthesis was reduced precipitously [44]. In earlier studies we showed that a static load of 1 MPa on human articular cartilage explants in vitro resulted in an increased expression of IL-1β [45] and PGE₂ [15], though cyclic loading produced no measurable change in either IL-1β or PGE₂ suggesting that this is a pathological response. In this study, we show that physical injury to cartilage following impact results in a significant increase in PGE₂.

The increase in the percentage of apoptotic cells was similar to that we measured previously with human cartilage using the same drop-tower model [46]. The role of PGE₂ in promoting apoptosis, however, remains unclear. Addition of exogenous PGE₂ to bovine articular chondrocytes in vitro has been shown to cause apoptosis through a cAMP-dependent pathway [29]. However in human chondrocytes from OA cartilage, Notoya et al. [47] found that PGE₂ had no effect on chondrocyte apoptosis itself, but the prostanoid enhanced apoptosis induced by exogenous nitric oxide and this effect could be prevented by COX-2 inhibition. In contrast, PGE₂ has been reported to protect chondrocytes from apoptosis induced by actinomycin-D [48]. In addition, PGE₂ is a chondrocyte growth inhibitor that requires NO for its production [49], and both PGE₂ and NO are downstream mediators of IL-1. The partial, dose-independent reduction we found suggests that a cofactor role for PGE₂ is possibly more likely than a direct effect. Further studies are required to investigate the role of NO and IL-1β.

Hashimoto et al. [21] linked chondrocyte apoptosis to matrix destruction. In this study however, the inhibition of apoptosis by the addition of celecoxib or indomethacin was not found to reduce the amount of GAGs, the breakdown products of cartilage proteoglycans, lost from loaded explants. This result is similar to that we previously found, culturing human articular cartilage explants with a broad spectrum caspase inhibitor (Z-VAD-FMK) reduced the percentage of impact-induced apoptotic chondrocytes but was unable to reduce the amount of GAGs released into the medium following impact [50]. Since articular cartilage is not vascularised and does not con-
tain mononuclear phagocytes, there is no apparent mecha-
nism for removal of apoptotic bodies following chondrocyte
apoptosis. It has been shown that these apoptotic bodies
express properties that contribute to pathologic matrix
destruction [27]. Therefore, although matrix degradation, as
measured by GAG release, was unchanged in this study, COX
inhibition may still have chondroprotective effects by reducing
the percentage of impact-induced apoptotic cells remaining in
the tissue (and, therefore, there would be fewer potentially
destructive apoptotic bodies). Together, though, these studies
indicate that apoptosis alone is not driving matrix breakdown
and that, once started, degradative enzymic activity may not be
under direct cellular control. This may be because these
enzymes are sequestered extracellularly in inactive forms and
activation leads to a positive feedback pathway that is then out
of direct control of the cells. Alternatively, enzymic activation
is controlled by a different signalling pathway, though this then
raises the question as to why the remaining cells cannot rec-
ognise this activity and inhibit it? The cells have been shown
to be able to increase their levels of matrix biosynthesis follow-
ing impact-induced damage [7] so perhaps matrix breakdown is
part of a repair response to try to remove damaged tissue and
replace it. Assuming, however, that it is important clinically
to reduce matrix degradation, a two-pronged approach to
treating damaged tissue would then be required; one agent
to rescue the cells from apoptosis and another to inhibit enzymic
degradation. The complexity of these mechanisms requires
further investigation, in particular addition of exogenous PGE2
and subsequent measurement of proteoglycan synthesis in this
system would demonstrate any anabolic effects. However,
we have shown that inhibiting PGE2 in impact-damaged carti-
lage at least partially prevented the increase in apoptotic cells
otherwise found after 6 days.

Both celecoxib and indomethacin could abolish the increase in
PGE2. Celecoxib is 375 times more selective for COX-2 than
COX-1 [51] whereas indomethacin is generally considered to
be non-selective. Several studies have shown that COX inhib-
itors have an effect on cartilage metabolism. COX-2 inhibition
has no direct effect on normal, healthy cartilage, but in the
presence of IL-1β or TNFα it restores proteoglycan turnover
[52]. Additionally, Hajjaji et al. [53] found that celecoxib had a
favourable effect on the metabolism of proteoglycans and
hyaluronic acid in samples of OA cartilage in vitro. Non-selective
NSAIDs have differing effects on cartilage metabolism. Some
stimulate, some have no effect, and others – including
indomethacin – inhibit matrix synthesis [54,55]. Mastbergen et
al. [38] have shown that in OA cartilage, NSAIDs with low
COX-2/COX-1 selectivity exhibit adverse effects whereas
high COX-2/COX-1 selective NSAIDs either had no effect or
had reparative properties. Since in our study the non-selective
NSAID indomethacin inhibited impact-induced apoptosis,
future experiments with an experimental selective COX-1
inhibitor (i.e. SC-560) may be of use to investigate the precise
role of COX-1 in this impact model. In patients, selective COX-
2 inhibition would appear to confer beneficial effects on artic-
ular cartilage metabolism while avoiding the harmful effects of
COX-1 inhibition, such as gastric irritation and inhibition of
matrix synthesis, though possible cardiovascular effects of
COX-2 inhibition have yet to be resolved.

Conclusion
This study has shown that an impact load on articular cartilage
results in a marked increase in PGE2 synthesis. This increase
could be abolished by both the selective COX-2 inhibitor,
celecoxib, and by non-selective indomethacin. Chondrocyte
apoptosis, induced by impact, was also reduced by COX-2
inhibition. No change was observed in the release of GAGs
from the explants in the presence of these inhibitors, however,
suggesting that the COX/PGE2 pathway is not directly
responsible for cartilage breakdown following traumatic injury.
The inhibition by COX inhibitors of PGE2 release following
trauma may provide an opportunity for early clinical interven-
tion to reduce cell death from apoptosis. Our in vitro study
suggests that it is unlikely, however, that COX-2 inhibition
alone would slow down or prevent the development of sec-
ondary osteoarthritis.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
JEJ designed the study, performed the experimental work, ana-
lysed the data and drafted the manuscript. RMA conceived of
the study, participated in its design and coordination and
revised the manuscript. All authors read and approved the final
manuscript.

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References
1. Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag
MJ: Joint injury in young adults and risk for subsequent knee
and hip osteoarthritis. Ann Intern Med 2000, 133:321-328.
2. Lohmander LS, Hoenner LA, Dahlberg L, Roos H, Björnsson S,
Lark MW: Stromelysin, tissue inhibitor of metalloproteinases
and proteoglycan fragments in human knee joint fluid after
injury. J Rheumatol 1993, 20:1382-1388.
3. Dahlberg L, Fridén T, Roos H, Lark MW, Lohmander LS: A longi-
tudinal study of cartilage matrix metabolism in patients with
cruciate ligament rupture – synovial fluid concentrations of
aggrecan fragments, stromelysin-1 and tissue inhibitor of
metalloproteinase-1. Br J Rheumatol 1994, 33:1107-1111.
4. Lohmander LS, Yoshihara Y, Roos H, Kobayashi T, Yamada H,
Shinmei M: Procollagen II C-propeptide in joint fluid: changes
in concentration with age, time after knee injury, and
osteoarthritis. J Rheumatol 1996, 23:1765-1769.
5. Hauk RC, Ide TM, Decamp CE: Mechanical responses of the
rabbit patello-femoral joint to blunt impact. J Biomech Eng
1995, 117:402-408.
6. Mrosek EH, Lahm A, Ergeleit C, Uhl M, Kurz H, Eissner B, Schage-
mann JC: Subcondral bone trauma causes cartilage matrix
degeneration: an immunohistochemical analysis in a canine model. Osteoarthritis Cartilage 2006, 14:171-178.
7. Jeffrey JE, Thomson LA, Aspden RM: Matrix synthesis and synthesis following a single impact load on articular cartilage in vitro. Biochim Biophys Acta 1997, 1334:223-232.
8. Attur M, Abramson SB, Amín AR, Patel IR, Patel RN: Autocrine production of IL-1 beta by human osteoarthrosis-affected cartilage and differential regulation of endogenous nitric oxide, IL-6, prostaglandin E2, and IL-8. Proc Assoc Am Physicians 1998, 110:65-72.
9. Saha N, Moldovan F, Tardiff G, Pelletier JP, Martel-Pelletier J, Interleukin-1-beta-converting enzyme/caspase-3 in human osteoarthrosis-affected cartilage: localization and role in the maturation of interleukin-1-beta and interleukin-18. Arthritis Rheum 1999, 42:1577-1587.
10. MacFarland AK, Ewen SB, Hoyland JA, Aigner T, Keenan G, Hashimoto S, Ochs RL, Komiya S, Lotz MK: Localization and characterization of the response of chondrocytes to mechanical loading using in situ hybridization. Matrix Biol 1994, 14:378.
11. Xie W, Chipman JG, Robertson DL, Erikson RL, Simons DL: Expression of a mitogen-responsive gene encoding prostaglandin H2-receptor mRNAs in cartilage. Proc Natl Acad Sci USA 1991, 88:2692-2696.
12. Vane JR, Bakhle YS, Botting RM: Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998, 38:97-120.
13. Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, Kosi A, Tripp CS: Cyclooxygenase-2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthrosis explants. Arthritis Rheum 2002, 46:1789-1803.
14. Martel-Pelletier J, Pelletier JP, Fahmi H: Cyclooxygenase-2 and prostaglandins in articular tissues. Sem Arthritis Rheum 2003, 33:155-167.
15. MacFarland AK, Johnstone AJ, Gordon MJ, Dutta-Roy AK, Aspden RM: Mechanical load stimulates prostaglandin E2 synthesis by articular cartilage chondrocytes in vitro. Matrix Biol 1996, 15:172.
16. Takahashi T, Ogawa Y, Kitaoka K: Selective COX-2 inhibitor regulates the MAP kinase signaling pathway in human osteoarthritic chondrocytes after induction of nitric oxide. J Mol Med 2005, 16:213.
17. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR: Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 1993, 90:11693-11697.
18. Loo K, Takahashi K, Amiel D, Tripp CS: Nitric oxide-induced apoptosis of bovine articular cartilage induces chondrocyte apoptosis. Arthritis Rheum 1998, 41:1266-1274.
19. Héraud F, Heraud A, Harmand MF: Apoptosis in normal and osteoarthrosis human articular cartilage. Ann Rheum Dis 2000, 59:959-965.
20. Bianco F, Guitian R, Vasquez-Martel E, de Toro FJ, Galdo F: Osteoarthrosis chondrocytes die by apoptosis. Arthritis Rheum 1998, 41:284-289.
21. Kimata K, Ochs RL, Komiya S, Lotz M: Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthrosis. Arthritis Rheum 1998, 41:1632-1638.
22. Loeving AM, James IE, Levenston ME, Badger AM, Frank EH, Kurz B, Nuttall ME, Hung HH, Blake SM, Grodinsky AJ, Lark MW: Inhibition of prostaglandin synthesis by cloproxifen-induced apoptosis in human cartilage. J Cell Biochem 2000, 87:205-212.
23. Chen GT, Burton-Wurster N, Borden C, Hueffer K, Bloom SE, Lust G: Chondrocyte necrosis and apoptosis in impact damaged articular cartilage. J Orthop Res 2001, 19:307-311.
24. D’Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW: Cartilage injury induces chondrocyte apoptosis. J Bone Joint Surg Am 2001, 83-A Suppl 2:19-21.
25. D’Lima DD, Hashimoto S, Chen PC, Colwell CW, Lotz MK: Human chondrocyte apoptosis in response to mechanical injury. Osteoarthritis Cartilage 2001, 9:712-719.
26. Levin A, Burton-Wurster N, Chen CT, Lust G: Intercellular signaling as a cause of cell death in cyclically impacted cartilage explants. Osteoarthritis Cartilage 2001, 9:702-711.
27. Lotz M, Hashimoto S, Kuhn K: Mechanisms of chondrocyte apoptosis. Osteoarthritis Cartilage 1999, 7:389-391.
28. Bratton SB, MacFarlane M, Cain K, Cohen GM: Protein complexes activate distinct caspase cascades in death receptor and stress-induced apoptosis. Exp Cell Res 2000, 258:97-103.
29. Miwa M, Saura R, Hirata S, Hayashi Y, Mizuno K, Itoh H: Induction of apoptosis in bovine articular chondrocyte by prostaglandin E2 through cAMP-dependent pathway. Osteoarthritis Cartilage 2000, 8:17-24.
30. Jeffrey JE, Gregory DW, Aspden RM: Matrix damage and chondrocyte viability following a single impact load on articular cartilage. Arch Biochem Biophys 1995, 322:87-96.
31. Burgin LV, Aspden RM: A drop tower for controlled impact testing of biological tissues. Med Eng Phys 2007, 29:525-530.
32. Fornier B, Weinberg J, Piuskiew DS, Musikons MA, Fink C, Guik L: Induction of cyclooxygenase-2 by mechanical stress through a nitric oxide-regulated pathway. Osteoarthritis Cartilage 2002, 10:792-798.
33. Jeffrey JE, Aspden RM: The biophysical effects of a single impact load on human and bovine articular cartilage. Proc IMechE H: J Eng Med 2006, 220:677-686.
34. Helleberg L: Clinical pharmacokinetics of indomethacin. Clin Pharmacokinet 1981, 6:245-258.
35. Vandale RW, Buttle J, Barrett AB, Jr: Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylen blue. Biochim Biophys Acta 1986, 883:173-177.
36. Stone JE, Akhtar N, Botchway S, Pennock CA: Interaction of 1,9-dimethylmethylen blue with glycosaminoglycans. Ann Clin Biochem 1994, 31:147-152.
37. Mastbergen SC, Bijlsma JW, Lafeber FP: Selective COX-2 inhibition is favorable to human early and late-stage osteoarthritic cartilage: a human in vitro study. Osteoarthritis Cartilage 2005, 13:319-326.
38. Mastbergen S, Jansen N, Bijlsma J, Lafeber F: Differential direct effects of cyclo-oxygenase-1/2 inhibition on proteoglycan turnover of human osteoarthritic cartilage: an in vitro study. Arthritis Res Ther 2008, 8:R2.
39. Tchetina EV, Antoniou J, Tanzer M, Zukor DJ, Poole AR: Transforming growth factor-beta 2 suppresses collagen cleavage in cultured human osteoarthritic cartilage, reduces expression of genes associated with chondrocyte hypertrophy and degradation, and increases prostaglandin E2 production. Am J Pathol 2006, 168:131-140.
40. Mitrovic D, McCall E, Dray F: The in vitro production of prostanooids by cultured bovine articular chondrocytes. Prostaglandins 1982, 23:17-28.
41. Di Battista JA, Dore S, Morin H, Ye P, Pelletier JP, Martel-Pelletier J: Prostaglandin E2 stimulates insulin-like growth factor binding protein-4 expression and synthesis in cultured human articular chondrocytes: possible mediation by Ca++-calmodulin regulated processes. J Cell Biochem 1997, 65:408-419.
42. Lowe GN, Fu YH, McDougall S, Polendo R, Williams A, Benya PD, Lowe P, et al.: Effects of prostaglandins on deoxyribonucleic acid and aggrcan synthesis in the RCJ 3.1C5.18 chondrocyte cell line: role of second messengers. Endocrinology 1996, 137:2208-2216.
43. Di Battista JA, Pelletier JP, Cloutier JM, Martel-Pelletier J: Modulation of glucocorticoid receptor expression in human articular chondrocytes by cAMP and prostaglandins. J Rheumatol Suppl 1991, 27:102-105.
44. Di Battista JA, Dore S, Martel-Pelletier J, Pelletier JP: Prostaglandin E2 stimulates incorporation of proline into collagenase digestible proteins in human articular chondrocytes: identification of an effector autocrine loop involving insulin-like growth factor I. Mol Cell Endocrinol 1996, 123:27-35.
45. MacFarland AK, McGuan GE, Hoyland JA, Ewen SB, Raiston SR, Aspden RM: The effect of mechanical load on synovial gene expression in bovine and human articular cartilage analysed by messenger RNA phenotyping. Int J Exp Pathol 1995, 76:A26.
46. Jeffrey JE, Li LLM, Aspden RM: The role of the proteolytic enzyme, cathepsin B, in articular cartilage degradation following an impact load. Osteoarthritis Cartilage 2004, 12:548.
47. Notoyo K, Jovanicov D, Rebol P, Martel-Pelletier J, Mineau F, Pelletier JP: The induction of cell death in human osteoarthritic chondrocytes by nitric oxide is related to the production of prostaglandin E2, via the induction of cyclooxygenase-2. J Immunol 2000, 165:3402-3410.
48. Lopez-Armada MJ, Carames B, Lires-Dean M, Cillero-Pastor B, Ruiz-Romero C, Galdo F, et al.: Cytokines, tumor necrosis factor-α and interleukin-1β, differentially regulate apoptosis in osteoarthritis cultured human chondrocytes. *Osteoarthritis Cartilage* 2006, 14:660-669.

49. Blanco FJ, Lotz M: IL-1-induced nitric oxide inhibits chondrocyte proliferation via PGE₂. *Exp Cell Res* 1995, 218:319-325.

50. Bennett KA, Jeffrey JE, Aspden RM: Chondrocyte apoptosis in human articular cartilage following an impact load. *Osteoarthritis Cartilage* 2003, 11:S17.

51. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Grantee MJ, Lee LF, Malecha JW, Miyashiro JM, et al.: Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). *J Med Chem* 1997, 40:1347-1365.

52. Mastbergen SC, Lafeber FPJG, Bijlsma JWJ: Selective COX-2 inhibition prevents proinflammatory cytokine-induced cartilage damage. *Rheumatology* 2002, 41:801-808.

53. Hajjaji H, Marcelus 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, 30:2444-2451.

54. McKenzie LS, Taylor TK, Horsburgh BA, Ghosh P: Effect of anti-inflammatory drugs on sulphated glycosaminoglycan synthesis in aged human articular cartilage. *Ann Rheum Dis* 1976, 35:487-497.

55. Dingle JT: The effects of NSAID on the matrix of human articular cartilages. *Z Rheumatol* 1999, 58:125-129.