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The chondroprotective effect of selective COX-2 inhibition in osteoarthritis: *ex vivo* evaluation of human cartilage tissue after *in vivo* treatment

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Summary

Objective: Recent *in vitro* studies showed that celecoxib, a selective cyclooxygenase (COX)-2 inhibitor, protects human osteoarthritic cartilage tissue from degeneration. The objective was to substantiate these beneficial effects in an *in vivo* (clinical) study with celecoxib treatment of patients with severe knee osteoarthritis (OA) and subsequent evaluation of cartilage tissue *ex vivo*.

Methods: Patients with knee OA were treated 4 weeks prior to total knee replacement surgery with either celecoxib 200 mg b.d., indomethacin 50 mg b.d., or received no treatment. During surgery cartilage and synovium were collected and analyzed in detail *ex vivo*.

Results: When compared to non-treated patients, patients treated with celecoxib showed significant beneficial effects on proteoglycan synthesis, -release, and -content, confirming the *in vitro* data. In the indomethacin group, no significant differences were found compared to the control group. On the contrary, a tendency towards a lower content and lower synthesis rate was found. In the treated groups prostaglandin-E_2 levels were lower than in the control group, indicating COX-2 inhibition. *Ex vivo* release of interleukin-1β (IL-1β) and tumour necrosis factor-α by synovial tissue was decreased by treatment with celecoxib, whereas in the indomethacin group only IL-1β release was decreased.

Conclusion: Using this novel approach we were able to demonstrate an *in vivo* generated chondrobeneficial effect of celecoxib in patients with end stage knee OA.

Key words: Osteoarthritis, Celecoxib, Selective COX-2 inhibitor, Cartilage.

Introduction

Osteoarthritis (OA) is a slowly progressive degenerative joint disorder, with a high prevalence, gradual degeneration of articular cartilage, peri-articular bone changes and secondary synovitis. Pain is the most important symptom in patients with OA[1–4]. The first choice for pharmacological pain management in patients with OA, considering the guidelines of the American College of Rheumatology (ACR)[5], is still acetaminophen. But more recent studies indicate that, especially in patients with severe pain, non-steroidal anti-inflammatory drugs (NSAIDs) are superior[6]. Additionally, patients with OA show a greater preference for NSAIDs[7–9]. More recently, the Osteoarthritis Research Society International (OARSI) Guidelines Committee agrees that NSAIDs, both non-selective and selective, have a major role in the management of OA[10].

Anti-inflammatory and analgesic effects of NSAIDs are mainly due to their ability to suppress cyclooxygenase (COX), an enzyme involved in the production of prostaglandins. The clinical efficacy and side-effects with respect to gastrointestinal (GI) problems of NSAIDs are mostly well understood[11,12]. According the OARSI Guidelines Committee selective NSAIDs appear to be safer with respect to GI toxicity and tolerance than non-selective NSAIDs[10]. Cardiovascular side-effects of the second generation NSAIDs, the selective COX-2 inhibitors (Coxibs), became evident more recently[13–16]. But also for the conventional NSAIDs this has always been a concern[17,18]. Adverse effects of selective COX inhibitors on bone turnover/repair are still subject of study[19].

Another aspect of NSAIDs, still ignored by many, is their direct possible (adverse) effects on cartilage. Direct effects of NSAIDs on cartilage may be of importance in treatment of joint diseases, specifically in prolonged treatment of joint disease in which inflammation is only mild and secondary, as in OA. These direct effects should be considered in addition to GI effects and cardiovascular effects when prescribing NSAIDs in clinical practice. Specifically in case of chronic use in not primarily inflammation mediated joint disease.

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Data about these direct effects of NSAIDs on cartilage are not consistent. Adverse effects have been reported frequently but also beneficial or neutral effects have been reported *in vitro* and in animal *in vivo* studies[20–22]. A frequently used NSAID that has been studied in the past regarding its direct effects on cartilage is indomethacin. Indomethacin shows principally negative results on the biochemical parameters of cartilage *in vitro*[21,23] and animal studies[24,25], but there are also studies that could not find any effect of indomethacin on cartilage[26,27].

Data on the selective COX-2 inhibitors are scarce. Recent *in vitro* data on celecoxib have shown positive effects on cartilage of OA patients[28–31]. These studies showed that celecoxib has favourable effects on the turnover of proteoglycans of OA cartilage, especially by diminishing the loss of proteoglycans from the matrix and increasing the retention of newly formed proteoglycans. To a lesser extend the synthesis of proteoglycans is influenced beneficially.

The direct effects of NSAIDs on cartilage cannot be studied easily in clinical trials and, therefore, they are generally ignored in clinical practice. Moreover, effects of NSAIDs on inflammation[32] shade their direct effects on cartilage. In addition (intrinsic) cartilage changes, catabolic and anabolic, are generally very slow processes in OA and evaluation of cartilage degeneration by imaging techniques is still hampered by the limited sensitivity of these techniques. Therefore, we used an approach in which *in vivo* treatment is combined with *ex vivo* evaluation of the cartilage tissue. By treating patients with NSAIDs shortly before joint replacement surgery, significant amounts of cartilage tissue that have been exposed *in vivo* become available for *ex vivo* biochemical and histochemical analyses. This gives us the opportunity to study in a relatively short period, the effects of NSAIDs on OA cartilage treated *in vivo* by use of detailed *ex vivo* biochemical and histochemical analyses.

The aim of this study was to investigate the effects of treatment of patients with severe knee OA with celecoxib for 4 weeks, in comparison with the conventional NSAID indomethacin, and with no treatment, on the biochemical characteristics of the joint cartilage.

Methods

PATIENTS

Twenty-eight patients with severe knee OA, who were eligible for total knee replacement surgery, have been included between December 2004 and April 2005. Patients were treated at the University Medical Center of Utrecht (UMCU), the Netherlands ($n=15$); the Sint Franciscus Gasthuis Hospital in Rotterdam, the Netherlands ($n=9$) and the Diakonessenhuis Hospital in Utrecht, the Netherlands ($n=4$).

Exclusion criteria were a total knee replacement for other reasons than OA, patients with a history of GI bleedings or perforation, and patients with an increased risk for cardiovascular diseases (patients with a history of cardiovascular disease, with untreated hypertension, with angina pectoris, and patients on oral anti-coagulants). Patients already on NSAIDs had to stop their medication and switched to the study-medication after a wash-out period of 7 days. The study was conducted according to the declaration of Helsinki and received ethics approval in all centres. Each patient gave written informed consent before participating in the study.

STUDY DESIGN

Patients with knee OA were randomized treated 4 weeks prior to knee replacement surgery with celecoxib 200 mg b.d. ($n=12$), indomethacin 50 mg b.d. ($n=8$), or received no treatment ($n=8$; controls). Because of its platelet-inhibiting effect, indomethacin-usage had to be stopped 3 days prior to operation which was compensated for by a 3-day earlier start of medication and because of the increased risk for GI adverse effects with the use of indomethacin, all patients on this medication also received omeprazol (20 o.d. mg). Controls did not use NSAIDs according to their medical records and were informed explicitly not to take an occasional NSAID in the last 4 weeks prior to operation.

At joint replacement surgery, synovial tissue and cartilage with underlying bone were obtained from femoral condyles and tibial plateaus. This tissue was kept in phosphate buffered saline for less than 4 h and subsequently processed under laminar flow conditions at the UMCU. The investigators who performed the experiments and analysis were blinded to the patients' clinical data and medication use. All the cartilage present on the weight bearing area of the joint was cut aseptically from the underlying bone. The slices were cut into squares and weighted (range 5–15 mg, accuracy 0.1 mg). Twenty-four samples were randomly selected the remaining sample were discarded. Four of these randomly taken samples of each donor were fixed in 4% phosphate buffered formalin in 2% sucrose and stained with Safranin-O fast green–iron haematoxylin for histochemistry. The remaining 20 randomly taken cartilage samples of each donor were used for biochemical analysis of proteoglycan synthesis, -retention, -release, -content, prostaglandin-E_2 (PGE_2) production, and nitric oxide (NO) production. Additionally, four synovial tissue samples from each donor were taken for determination of synovial tissue derived pro-inflammatory/tissue destructive cytokine production and matrix metalloproteinase (MMP) activity.

HISTOCHEMISTRY

Four randomly taken cartilage samples were graded for features of OA, using the modified Mankin criteria[33,34]. Two observers blinded to the source of the cartilage graded the cartilage and the averages of the two observers and the four samples were taken as representative score of each donor.

PROTEOGLYCAN ANALYSIS

As a measure of the proteoglycan synthesis rate, the sulphate incorporation rate was determined over a 4-h period. $Na_2SO_4^{2-}$ (DuPont NEX-041-H, carrier free) 370 kBq was added to each 200 μl culture medium, consisting of Dulbecco's modified Eagle's medium (DMEM), supplemented with glutamine (2 mM), penicillin (100 IU/ml), streptomycin sulphate (100 μg/ml) ascorbic acid (0.085 mM) and 10% heat inactivated pooled human male AB^+ serum. Samples were equilibrated for 1 h before adding $^{35}SO_4^{2-}$. After the 4-h labelling period, the cartilage explants were washed three times for 45 min in culture medium under culture conditions and then cultured, individually in a 96-well round bottomed microtiter plate (200 μl culture medium/well 37°C, 5% CO_2 in air) for an additional period of 3 days. After this period cartilage and culture medium were separated, snap frozen, and stored at −20°C for no longer than 7 days.

For proteoglycan synthesis and -content, cartilage samples were digested (2 h, 65°C) in papain buffer as described before[35]. Papain digests were diluted to the appropriate concentrations and glycosaminoglycans (GAGs) were stained and precipitated with Alcian Blue dye solution[36]. The pellet obtained after centrifugation (9000*g*, 10 min) was washed once (sodium acetate (NaAc) buffer containing 0.1 M $MgCl_2$) and subsequently dissolved (sodium dodecyl sulphate (SDS)). The $^{35}S^-$ radioactivity of the sample was measured by liquid scintillation analysis after addition of Picofluor-40 (Packard). $^{35}SO_4^{2-}$ incorporation was normalized to the specific activity of the medium, labelling time, and wet weight of the cartilage samples. The proteoglycan synthesis rate is expressed as nanomoles of sulphate incorporated per hour per gram wet weight of the cartilage (nmol/h/g). Blue staining was quantified photometrically by the change in absorbance at 620 nm. Chondroitin sulphate (C4383; Sigma) was used as a reference. Values were normalized to the wet weight of the cartilage and expressed as milligrams of GAG per gram wet weight of cartilage (mg/g).

Release of newly formed proteoglycans, as a measure of retention of these proteoglycans, and total proteoglycan release was determined in culture medium. GAGs were precipitated from the 3-day culture medium and stained with Alcian Blue[36]. As a measure of the release of newly formed proteoglycans the ^{35}S-radio-labelled GAGs were measured by liquid scintillation analysis, normalized to the proteoglycan synthesis rate, and expressed as percentage release of newly formed proteoglycans. For the total release of proteoglycans blue staining was quantified photometrically by the change in absorbance at 620 nm (chondroitin sulphate used as a reference). Values were normalized to the GAG content of the explants and expressed as a percentage release.

PGE_2 AND NO DETERMINATION

PGE_2 and NO were determined in the *ex vivo* 3-day culture medium. PGE_2 was determined by Enzyme Immuno Assay (EIA, Caymann Chemical) and expressed as pg/ml/mg (wet weight) cartilage tissue. NO levels were determined using the standard Griess reaction and expressed as μM/mg (wet weight) of cartilage tissue.

SYNOVIAL TISSUE ANALYSIS

Four synovial tissue samples (192 ± 93 mg) from each donor were cultured for 3 days in 4 ml culture medium. The supernatants of these cultures