"Effects of tenoxicam and aspirin on the metabolism of proteoglycans and hyaluronan in normal and osteoarthritic human articular cartilage."

Manicourt, Daniel ; Druetz-Van Egeren, A ; Haazen, L. ; Nagant de Deuxchaisnes, C

Abstract
1. As nonsteroidal anti-inflammatory drugs may impair the ability of the chondrocyte to repair its damaged extracellular matrix, we explored the changes in the metabolism of newly synthesized proteoglycan (PG) and hyaluronan (HA) molecules produced by tenoxicam and aspirin in human normal cartilage explants and in osteoarthritic (OA) cartilage from age-matched donors. 2. Explants were sampled from the medial femoral condyle and were classified by use of Mankin's histological-histochemical grading system. Cartilage specimens were normal in 10 subjects, exhibited moderate OA (MOA) in 10 and had severe OA (SOA) in 10. 3. Cartilage explants were pulsed with [3H]-glucosamine and chased in the absence and in the presence of either aspirin (190 micrograms ml-1) or tenoxicam (4-16 micrograms ml-1). After papain digestion, the labelled chondroitin sulphate ([3H]-PGs) and HA([3H]-HA) molecules present in the tissue and media were purified by anion-exchange chromatography. 4. In normal cartilag...

Document type : Article de périodique (Journal article)

Référence bibliographique

Available at:
http://hdl.handle.net/2078.1/13398
[Downloaded 2019/03/26 at 13:25:03 ]
Effects of tenoxicam and aspirin on the metabolism of proteoglycans and hyaluronan in normal and osteoarthritic human articular cartilage

**Daniel Henri Manicourt, Anne Druetz-Van Egeren, Ludo Haazen & Charles Nagant de Deuxchaisnes**

ICP, *Connective Tissue Group and Department of Rheumatology, Saint-Luc University Hospital, University of Louvain in Brussels, Brussels, Belgium and the **Produits Roche S.A., Brussels, Belgium*

1 As nonsteroidal anti-inflammatory drugs may impair the ability of the chondrocyte to repair its damaged extracellular matrix, we explored the changes in the metabolism of newly synthesized proteoglycan (PG) and hyaluronan (HA) molecules produced by tenoxicam and aspirin in human normal cartilage explants and in osteoarthritic (OA) cartilage from age-matched donors.

2 Explants were sampled from the medial femoral condyle and were classified by use of Mankin's histological-histochemical grading system. Cartilage specimens were normal in 10 subjects, exhibited moderate OA (MOA) in 10 and had severe OA (SOA) in 10.

3 Cartilage explants were pulsed with [3H]-glucosamine and chased in the absence and in the presence of either aspirin (190 μg ml⁻¹) or tenoxicam (4–16 μg ml⁻¹). After papain digestion, the labelled chondroitin sulphate ([3H]-PGs) and HA-[3H]-HA molecules present in the tissue and media were purified by anion-exchange chromatography.

4 In normal cartilage as well as in explants with MOA and SOA aspirin reduced more strongly PG and HA synthesis than the loss of [3H]-HA and [3H]-PGs.

5 In normal cartilage, tenoxicam did not affect PG metabolism whereas it reduced HA synthesis in a dose-dependent manner and did not change or even increased the net loss of [3H]-HA. In contrast, in OA cartilage, tenoxicam produced a stronger reduction in the loss of [3H]-PGs than in PG synthesis and this decrease occurred at lower concentrations in cartilage with SOA (4–8 μg ml⁻¹) than in cartilage with MOA (8–16 μg ml⁻¹). In cartilage with MOA, the metabolic balance of HA was unaffected by tenoxicam whereas in cartilage with SOA, the drug decreased the loss of [3H]-HA and concomitantly did not change or even increased HA synthesis.

6 The data obtained in short-term *in vitro* cultures indicate that aspirin may produce OA-like changes in normal cartilage and is likely to worsen the disease process in OA tissue. On the other hand, although tenoxicam may reduce the HA content of normal cartilage, and, in so doing, may produce OA-like lesions, this drug should not *per se* accelerate joint failure in OA.

**Keywords:** Nonsteroidal anti-inflammatory drugs; aspirin; tenoxicam; aggrecan; hyaluronan; normal cartilage; osteoarthritic cartilage

---

**Introduction**

Proteoglycans (PGs) and hyaluronan (HA) play an important role in the macromolecular organization and in the biomechanical properties of articular cartilage. PGs provide the articular tissue with its elasticity and stiffness in compression whereas the long strands of HA interact specifically with PG molecules to form supramolecular aggregates of very large size which are firmly immobilized within the collagen meshwork (Lohmander, 1988). PG aggregation also increases dramatically the rheological properties of PG molecules and, in so doing, affects the dynamic behaviour of cartilage in compression (Hardingham *et al.*, 1987). Therefore, any decrease in the tissue concentration of PG and HA, as occurs in osteoarthritis (OA), compromises the functional properties of cartilage.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed in patients suffering from rheumatological disorders and they undeniably produce relief of pain and improvement of joint mobility. The use of these drugs might however be detrimental to the joints. Effective pain relief could indeed lead to overse of a disabled joint. Further *in vitro* and *in vivo* studies have shown that some NSAIDs inhibit the synthesis of articular cartilage PGs whereas others do not (Brandt, 1987; Muir *et al.*, 1988; Howell *et al.*, 1991). This differential effect of NSAIDs on cartilage metabolism are most relevant to clinical practice as any drug that suppresses PG synthesis and impairs the ability of the chondrocyte to repair its damaged extracellular matrix, could potentially accelerate the breakdown of the articular tissue. On the other hand, although HA plays a central structural role in the macromolecular organization of PGs, the possible effects of NSAIDs on the metabolism of this glycosaminoglycan have not yet focussed any investigative attention.

Tenoxicam is a thienothiazine derivative of the oxiaim class. Although this drug displays good efficacy and tolerability in therapy for rheumatic disorders (Kirchheimer *et al.*, 1982; Gonzalez & Todd, 1987), knowledge of its possible effects on the metabolism of articular cartilage is still fragmentary (Franchimont *et al.*, 1989). We therefore investigated the action of tenoxicam on the metabolism of newly synthesized PGs and HA molecules in explant cultures from human normal and OA cartilage. Results were compared with those obtained with aspirin.

---

1 Author for correspondence at: Department of Rheumatology, UCL 53.90, Avenue Mounier, 1200 Bruxelles, Belgium.
Methods

Patients' evaluation and cartilage tissue sampling

Thirty subjects were included in the study. Exclusion criteria were septicaemia, connective tissue disorders, rheumatoid arthritis, crystal deposition diseases, known hereditary or congenital defects, immobilization for several weeks as well as treatment with corticosteroids and cytotoxic drugs.

Full thickness cartilage was sampled from the mediolateral condyle of the knee within 24 h postmortem and cartilage specimens were brought into sterile phosphate-buffered saline solutions (PBS) for transport to culture facilities.

For each individual, 3 cartilage slices were taken at random from the pool of sampled tissue and processed for the histological-histochemical grading system devised by Mankin et al. (1971). The mean score of the 3 cartilage slices was retained for each donor.

Articular cartilage was normal in 10 subjects whose mean ± s.d. age was 53.9 ± 4.8 years (range 46–61). Mankin's grading system ranged from 2–5 in 10 other individuals who were classified as having moderate osteoarthritis (MOA); their mean ± s.d. age was 54.4 ± 4.7 years (range 49–65) and none of the donors were known to have overt clinical manifestations of OA. In contrast Mankin's grading system ranged from 6–9 in 10 other subjects who were labelled as having severe OA (SOA). Their mean ± s.d. age was 56.8 ± 5.4 years (range 51–65): 8 out of these 10 donors had clinical manifestations of OA and were taking either analgesics (paracetamol in 2, dextropropoxyphen HCl in 2) and/or NSAIDs (diclofenac in 3, ibuprofen in 2, Naproxen in 2) on an irregular basis.

General culture procedures

Tissue specimens obtained from each donor were cut into pieces of 3–6 mg in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (5,000 i.u. ml\(^{-1}\)) and streptomycin (5,000 \(\mu\)g ml\(^{-1}\)) the tissue was washed 3 times with this culture medium and aspirated free from liquid. Cartilage pieces were taken at random, weighed and distributed into the different wells of multiwell culture plates (typically between 30 and 60 mg tissue/well). Extra pieces were not cultured but stored at -20°C in order to assess the initial PG and HA contents of the cartilage. Culture medium supplemented with 20% (v/v) foetal calf serum (culture medium A) was then added to each well and the culture plates were placed in a 90%:10% (v/v) air:CO\(_2\) humidified incubator for 48 h at 37°C.

For each experiment the cartilage from one individual was used and tissue cultures were conducted in triplicate: that is, for the control culture as well as for each NSAID concentration, 3 cartilage explants were cultured separately. Reported values are the mean of the triplicate cultures.

Pulse studies

After 2 days of culture, the culture medium was aspirated and the explants were washed 3 times with 1 ml of DMEM. Explants were resuspended in culture medium A (1 ml 50 mg ml\(^{-1}\) tissue supplemented with \([\text{H}]\)-glucosamine (50 \(\mu\)Ci ml\(^{-1}\)) (culture medium B). To each well, a solution of NSAID dissolved in dimethylsulphoxide (DMSO) was added (10 \(\mu\)l ml\(^{-1}\) in culture medium) to achieve the following final concentrations: 4, 8 and 16 \(\mu\)g ml\(^{-1}\) for tenoxicam and 190 \(\mu\)g ml\(^{-1}\) for aspirin. Control cultures received DMSO that did not contain any NSAID (10 \(\mu\)l ml\(^{-1}\)). Culture wells were then incubated for 12 h.

Chase studies

After one day of culture in medium A, the cartilage pieces were aspirated free of medium, washed 3 times with 1 ml of DMEM, resuspended in culture medium B (1 ml 50 mg ml\(^{-1}\) tissue) and cultured for 12 h. After pulse labelling, the cartilage pieces were washed with DMEM and resuspended in culture medium A. DMSO alone or the appropriate NSAID dissolved in DMSO was added to each well (10 \(\mu\)l ml\(^{-1}\)) as stated above and a non-radioactive chase period was conducted for 24 h.

Isolation and purification of hyaluronan and proteoglycans

At the end of the pulse labelling and non-radioactive chase periods, the culture media were removed and the cartilage pieces were washed with 0.15 M sodium chloride/0.05 M sodium acetate, pH 6.0 (buffer A). Media and corresponding washes were combined. Bovine nasal AlID1D1 PGs (500 pg ml\(^{-1}\)) and hyaluronan (10 pg ml\(^{-1}\)) were added. The mixtures were dialysed against buffer A and then incubated with papain (10 \(\mu\)g ml\(^{-1}\)) for 24 h at 60°C. Cartilage specimens were resuspended in buffer A; papain was added to each vial (0.1 mg ml\(^{-1}\)) and the tissues were digested for 24 h at 60°C.

Aliquots were sampled for hydroxyproline determinations or subjected to ion-exchange chromatography on Econo-Pak Q columns.

The Econo-Pak Q column (5 ml) was pre-equilibrated in buffer A at a flow rate of 2 ml min\(^{-1}\). Aliquots of papain-digested samples (100–200 \(\mu\)l for tissue digests and 0.5–1 ml for medium digests) were adjusted to 2 ml with buffer A and applied to the column that was then washed with 6 column volumes of buffer A. Anionic macromolecules were eluted from the column with 0.5 ml 0.5 M NaCl, hyaluronic acid molecules were eluted with 0.5 ml 1.0 M NaCl and hyaluronan (1961) was eluted with 1.0 ml 2.0 M NaCl. For each sample (tissue and media) fractions containing radio-labelled peaks were separated pooled, heated at 80°C for 10 min and applied to a column (0.7 x 50 cm) of Sephadex G-50 equilibrated in 0.1 M ammonium bicarbonate buffer, pH 8.5. In each case more than 90% of the radio-labelled material present in both peaks A and B eluted at the excluded volume of the G-50 column. Therefore, aliquots of peaks A and B were digested with Streptomyces hyaluronidase (0.1 u/ml) aliquots in 0.05 M Na acetate pH 5.0, 6 h at 60°C and chondroitinase ABC (0.5 u/ml) aliquots in 0.1 M Tris-fluoride, pH 8.0, 6 h at 37°C and the digestion products were applied to the G-50 column. Indeed Streptomyces hyaluronidase has an absolute specificity for HA and produces tetra- and hexasaccharides which are eluted in the included volume of the G-50 column. On the other hand, the digestion products of chondroitinase ABC are also included into the G-50 column but, in contrast to Streptomyces hyaluronidase, chondroitinase ABC digests both HA and chondroitin chains. Accordingly, the amount of [\(\text{H}\)]-HA present in each peak was calculated by multiplying the total radioactivity of the peak by the relative percentage of radio-labelled material that was sensitive to Streptomyces hyaluronidase whereas the amount of [\(\text{H}\)]-PG present in each peak was calculated by multiplying the total radioactivity of the peak by the relative percentage of radio-labelled material that was resistant to digestion with Streptomyces hyaluronidase and susceptible to digestion with chondroitinase ABC.

Analytical methods

Hydroxyproline was determined by the method of Woessner (1961) and hexuronic acid by the method of Bitter & Muir (1962). HA was quantified by a specific radioreceptor assay technique (Tengblad, 1980).
Materials

[3H]-glucosamine was from New England Nuclear Corporation (Brussels, Belgium). The scintillation liquid HiSafe-Optiphase II, purified hyaluronan (Healon) and Sephadex G-50 were from LKB-Pharmacia (Brussels, Belgium). Culture plates and media were from Gibco (Gent, Belgium). Chondroitin ABC lyase, Streptomyces hyaluronidase, twice crystallized papain and acetylsalicylic acid (aspirin) were from Sigma Chemical Company (St Louis, MO, U.S.A.). Econo-Pac Q cartridges were from Bio-Rad (Nazarath, Belgium). Dialysis membranes (mol. wt. cutoff: 3,500) were from Spectrum (Los Angeles, CA, U.S.A.). Tenoxicam was a gift from Roche (Basel, Switzerland). All other reagents were from Merck (Darmstadt, Germany).

Expression of results and statistics

The rate of biosynthesis of PG and HA was determined by the summation of [3H]-PG and [3H]-HA disintegrations per min (d.p.m.) found in papain-digested tissues and media at the end of the 12 h pulse labelling period and expressed as d.p.m. [3H]-PG and [3H]-HA per h and per mg of hydroxyproline. Indeed, the loss of hydroxyproline (and thus of collagen) from the normal and OA tissue specimens into the medium over a 72-h culture period was less than 5% of the amount present in cartilage pieces before culture.

At the end of the 24-h non-radioactive chase period, the total incorporation of [3H]-glucosamine into HA and PGs was determined by the summation of [3H]-HA and [3H]-PGs d.p.m. found in the media and corresponding papain-digested tissue specimens. The radiolabelled material that accumulated in the medium during this non-radioactive chase period represents not only degraded HA and PG molecules but also intact HA and PG molecules that were being synthesized at the end of the pulse period and that were not incorporated into the matrix and lost in the medium during the subsequent chase period. As the material present in the chase medium was not characterized in the present study, it is difficult to assess the respective proportion of these 2 processes and, accordingly, the radiolabelled material recovered in the medium during the 24-h chase period was not described as the result of catabolism but rather as net loss. This net loss was expressed as the percentage of total incorporated d.p.m. found in the medium samples of the 24-h period.

The statistical significance of the differences observed

![Figure 1](image1.png)

Figure 1 Distribution of values of total proteoglycan (PG) content, total hyaluronan (HA) content (a), rates of PG and HA synthesis (b) and net loss of newly synthesized PG ([3H]-PG) and HA ([3H]-HA) molecules (c) over a 24-h non-radioactive chase period in normal cartilage explants and in cartilage explants with moderate (M) and severe (S) osteoarthritis (OA) that were cultured in the absence of nonsteroidal anti-inflammatory drugs.
between groups was evaluated by the Mann-Whitney U test whereas in each group the significance of the differences in PG and HA metabolism in the presence of different NSAID concentrations were evaluated by the Wilcoxon signed-ranks test. P values <0.05 were considered as statistically significant.

Results

Biochemical and metabolic characterization of cartilage explants

The proteoglycan (PG) and hyaluronan (HA) contents of cartilage specimens were distributed over a wide range of values in the normal group and in the groups with moderate osteoarthritis (MOA) and severe osteoarthritis (SOA), as well (Figure 1, upper panels). Values found in normal explants (mean ± s.d. = 0.67 ± 0.07 and 0.04 ± 0.01 for the PG and HA contents, respectively) are in close agreement with those observed by Holmes et al. (1988) in normal articular tissue from the knee of age-matched subjects. The mean ± s.d. values of the PG and HA content were significantly higher in the normal group than in both OA groups (P<0.001). Further, the group with MOA had a significantly higher content of PG and HA than the group with SOA (0.53 ± 0.06 versus 0.41 ± 0.06 respectively for the PG content; U = 8; P<0.002; and 0.02 ± 0.01 versus 0.01 ± 0.01 respectively for the HA content; U = 7.5; P<0.002). This overall cartilage chemistry was not significantly affected by standard culture conditions and treatment with the NSAIDs examined over the 72-h period of culture (results not shown).

In the different pulse and chase experiments conducted in the absence and in the presence of NSAIDs, analysis of tissue and medium samples from normal and OA cartilage gave the following results: 50–70% of the labelled material present in peak A was sensitive to Streptomyces hyaluronidase and was thus identified as [3H]-HA. On the other hand, all the labelled material present in peak B was identified as chondroitin sulphate (and thus as proteoglycans) as it was consistently resistant to digestion with Streptomyces hyaluronidase and susceptible to complete digestion with chondroitinase ABC.

In the absence of NSAID, the rates of PG and HA synthesis were distributed over a wide range of values in the 3 groups (Figure 1, middle panels). There was no statistically significant difference in the rate of PG and HA synthesis between the group with moderate OA and the group with severe OA (57.30 ± 9.66 versus 60.30 ± 7.93, respectively for PG synthesis; U = 42.5; P>0.10 and 5.77 ± 0.92 versus 6.14 ± 0.81, respectively for HA synthesis; U = 36; P>0.10). The rates of PG and HA synthesis were however significantly higher (P<0.001) in the 2 OA groups than in the normal group (37.20 ± 7.83 for PG synthesis and 3.69 ± 0.72 for HA synthesis).

When labelled cartilage explants were cultured in the absence of drug the net loss of [3H]-HA and [3H]-PG molecules was distributed over a wide range of values in the 3 groups (Figure 1, bottom panels). Although the loss of [3H]-HA was significantly higher in the group with SOA than in the group with MOA (46.56 ± 5.79 versus 36.90 ± 4.41, respectively; U = 8.5; P<0.002) both OA groups did not differ in their loss of [3H]-PG (20.80 ± 6.61 for the group with MOA versus 28.00 ± 6.09 for the group with SOA). On the other hand, the loss of both [3H]-HA and [3H]-PG was significantly higher (P<0.001) in the 2 OA groups than in the normal group (12.0 ± 3.5 for the loss of [3H]-HA and 6.6 ± 3.2 for the loss of [3H]-PG).

These wide variations in composition and metabolism that, in each group, were displayed by the cartilage explants cultured in the absence of drug were likely to hamper the assessment of the effect of NSAIDs on PG and HA metabolism. Therefore, for each cartilage specimen of the 3 groups, the rates of PG and HA metabolism that were obtained in the presence of different concentrations of NSAID were divided by the values observed in the absence of drug to yield percentage changes.

Effects of tenoxicam and aspirin on the total synthesis of PG and HA

As shown in Figure 2a aspirin inhibited dramatically the total amounts (tissue + medium) of newly synthesized PG molecules in both normal and OA cartilage explants, whereas the effect of tenoxicam differed between normal and OA cartilage. Indeed in normal cartilage, there was no significant inhibition of PG synthesis over the range of tenoxicam concentrations examined. On the other hand, a significant reduction in PG synthesis was observed at a tenoxicam concentration of 8 μg ml−1 in cartilage with moderate OA and of 4 μg ml−1 in cartilage with severe OA. The strongest inhibition of PG synthesis induced by tenoxicam was however mild when compared to that observed in the presence of aspirin (84% versus 20% of control values, respectively).

In both normal and OA cartilage, tenoxicam inhibited the total amounts (tissue + medium) of newly synthesized HA molecules in a relatively dose-dependent manner over the range of concentrations examined (Figure 2b). The maximum inhibition produced by tenoxicam approximated 77% of control values. In contrast, the inhibition caused by 190 μg ml−1

![Figure 2](image-url)
of aspirin was quite stronger and ranged from 20% of control values in normal tissue to 32% of control values in OA specimens.

Effects of tenoxicam and aspirin on the relative amounts of newly synthesized PG and HA molecules incorporated within the cartilage matrix

During the pulse studies of normal and OA explants conducted in the absence of drug a relative proportion of newly synthesized PG and HA molecules were not incorporated within the extracellular matrix and were lost into the culture medium. Further, in both normal and OA groups, this relative loss of labelled molecules varied from one donor to another. Therefore, in each experiment the amounts of radiolabelled PG and HA (as expressed in d.p.m. h⁻¹ mg⁻¹ hydroxyproline) found in the explants at the end of the pulse-labelling period conducted in the presence of different concentrations of NSAIDs were divided by the content of corresponding labelled molecules observed in the control explants pulse-labelled in the absence of drug to yield percentage changes.

The changes in the tissue content of labelled PG obtained at each NSAID concentration are illustrated in Figure 3 (open columns). In normal cartilage the relative amounts of newly synthesized PGs remaining within the matrix at the end of the pulse labelling period were unaffected by tenoxicam over the range of concentration studied whereas a significant reduction was observed at a tenoxicam concentration of 16 µg ml⁻¹ in cartilage with moderate OA and of 8 µg ml⁻¹ in cartilage with severe OA. The maximum reduction in the cartilage content of labelled PG induced by tenoxicam approximated 85% of control values in contrast to the 12% of control values observed in the presence of aspirin.

The changes in the tissue content of labelled HA observed at each NSAID concentration are shown in Figure 4 (open columns). Tenoxicam had a quite different effect in normal and OA tissue. In normal cartilage 4 µg ml⁻¹ of tenoxicam had already produced a small but statistically significant reduction in the tissue content of labelled HA. This reduction became stronger with higher tenoxicam concentrations and reached approximately 76% of control values at a concentration of 16 µg ml⁻¹. In contrast, in cartilage with moderate OA the tissue content of newly synthesized HA, was unaffected by a tenoxicam concentration of 4 µg ml⁻¹. Higher tenoxicam concentrations were however able to reduce the labelled HA content of explants with moderate OA. This reduction was nevertheless significantly smaller (P<0.05) than that observed in normal explants at similar drug concentration. On the other hand, over the range of tenoxicam concentrations tested, there was no significant reduction in the content of labelled HA molecules remaining within the matrix of explants with severe OA and one could even

![Figure 3](image-url)

**Figure 3** Effects of different concentrations of tenoxicam and aspirin on the net loss of labelled proteoglycans (PGs) from the tissue during the 24-h non-radioactive chase period (solid columns) and on the relative amounts of newly synthesized PG molecules remaining within the matrix at the end of the pulse labelling period (open columns) in normal cartilage (a) and in explants with moderate (b) and severe (c) osteoarthritis. Results are expressed as the relative percentage of values found in explants cultured in the absence of drug. Comparison by the Wilcoxon signed-rank test: *P<0.05; **P<0.001.
observe a significant increase at a concentration of 4 μg ml⁻¹. Aspirin at a concentration of 190 μg ml⁻¹ reduced dramatically the tissue levels of labelled HA in both normal and OA explants. Although the levels observed in OA tissue were significantly higher than those observed in normal tissue ($P<0.001$), the values were nevertheless quite lower than those obtained in the presence of tenoxicam 16 μg ml⁻¹.

**Effects of tenoxicam and aspirin on the net loss of newly synthesized PG and HA**

In contrast to aspirin, tenoxicam did not induce any significant change in the net loss of labelled PGs from normal cartilage during the 24-h chase period (Figure 3, closed columns). On the other hand, in OA cartilage the reduction in the loss of labelled PGs varied with the concentration of tenoxicam and the severity of OA. Indeed, a significant reduction was observed at a concentration of 4 μg ml⁻¹ in explants with severe OA and of 8 μg ml⁻¹ in explants with moderate OA. Further, at each concentration examined, the effect of tenoxicam was always stronger in cartilage with severe OA than in cartilage with less advanced OA lesions.

In normal cartilage there was a significant increase in the loss of labelled HA at an aspirin concentration of 190 μg ml⁻¹ and at a tenoxicam concentration of 16 μg ml⁻¹ (Figure 4, closed columns). In contrast, in cartilage with moderate OA, 4 and 8 μg ml⁻¹ of tenoxicam reduced significantly the net loss of labelled HA and this reduction became even stronger ($P<0.001$) at a concentration of 16 μg ml⁻¹. In cartilage with severe OA, tenoxicam also inhibited the loss of labelled HA in a dose-dependent manner as there was a statistically significant difference ($0.008<P<0.02$) between the values obtained at the different drug concentrations. In both OA groups, 16 μg ml⁻¹ of tenoxicam reduced the loss of labelled HA to values similar to those found with 190 μg ml⁻¹ of aspirin.

**Discussion**

The present investigation is the first to describe the effect of tenoxicam and aspirin on the metabolism of proteoglycans (PGs) and hyaluronan (HA) in explants of human normal and osteoarthritic (OA) cartilage.

Cartilage composition and metabolism vary widely in the different topographical areas of the same joint, between the different joints of an individual, and between individuals, and both composition and metabolism change with age (Muir, 1986; Holmes et al., 1988). Accordingly, variations in the concentration and metabolism of both PG and HA were restricted by sampling the articular tissue from the same region of both normal and OA knees. Further, in the normal and OA groups, the donors had a very similar range of age distribution so that differences observed in response to
NSAIDs could be more closely related to the OA disease process rather than aging.

The aspirin and tenoxicam concentrations used in our culture system are similar to the synovial fluid levels of these drugs that have been observed in patients with OA of the knee (Palmski & Brandt, 1979; 1980; Day et al., 1991). It is however difficult to assess the actual effective concentration of drug acting on the tissue since this depends upon various factors such as the pH of the synovial fluid, the integrity of the surface of the cartilage, the partition coefficient of the drug and protein binding. Further, the final albumin concentration was lower in our culture system than in synovial fluid. Therefore it is likely that, at a tenoxicam level of 16 μg ml^-1, the concentration of the free drug in our culture media was greater than that present in the synovial fluid bathing cartilage in vivo.

Our study provides for the first time evidence that in normal articular cartilage both aspirin and tenoxicam are able concomitantly to reduce HA synthesis and increase the loss of newly synthesized HA molecules from the tissue. This negative metabolic balance of HA could eventually lead to OA like changes in the normal articular tissue as the OA cartilage matrix is characterized by a progressive depletion of its content in HA (Sweet et al., 1977; Manicourt & Pitta, 1988; Rizkalla et al., 1992). It should be however pointed out that the action of aspirin was considerably stronger than that of tenoxicam.

On the other hand, the 2 NSAIDs exhibited a quite different action on the HA metabolism of OA cartilage. Indeed, aspirin is likely to increase the HA depletion of the OA tissue as this drug reduced HA synthesis much more than the net loss of newly synthesized HA molecules. In contrast, in cartilage with severe OA, tenoxicam uncoupled HA synthesis from the loss of HA and produced a positive balance in HA synthesis. This metabolic effect on the synovial macrocyte is likely to decrease the HA depletion of the OA tissue and its exact mechanism should be elucidated in further studies as it might be of great biological and therapeutic significance in OA.

In agreement with previous reports (Palmski & Brandt, 1979; 1980; Muir et al., 1988), aspirin inhibited less strongly the net loss of PG than PG synthesis and the inhibition of PG synthesis was greater in OA cartilage than in normal cartilage. The reduction in the ability of chondrocytes to synthesize PGs was however more marked in the present study (15-25% of control values) than in previous investigations (60-70% of control values) conducted at similar drug concentrations (Brandt, 1987; Muir et al., 1988). It is possible that this difference reflects the fact that, in contrast to the present investigation, the previous studies were conducted on canine articular cartilage.

In contrast to aspirin, tenoxicam had no significant effect on the overall metabolism of PG in normal cartilage whereas in OA cartilage this NSAID produced a positive balance of PG metabolism. Indeed the reduction in the net loss of labelled PG molecules was always greater than the reduction in PG synthesis and cartilage with severe OA was more susceptible to this effect of tenoxicam than cartilage with moderate OA. This heightened susceptibility of pathological articular tissue has already been observed in vitro and in vivo with other NSAIDs (Brandt, 1987). Although its exact mechanism is unknown at present, data in the literature suggest that it is independent of the inhibition of the cyclooxygenase pathway by the drug and that the uptake of NSAID by cartilage is inversely related to the PG content of the matrix (Brandt, 1987). Accordingly, the decrease in the concentration of negatively charged PGs, which is proportional to the gravity of the OA process, would increase the permeability of the matrix to the aciddically charged NSAID.

The reduction in the net loss of PG produced by tenoxicam is quite consistent with a recent report (Vignon et al., 1992) showing that the proteoglycanase activity present in the cartilage of human OA femoral heads sampled at the time of total hip replacement was markedly reduced in the presence of this NSAID at a concentration of 4 μg ml^-1. It remains to determine whether this suppressive effect of tenoxicam on the loss of PGs is due either to an inhibition of the production of superoxide anions (Minta & Williams, 1985), or to a reduction in the synthesis and/or activation of proteolytic enzymes or to a stimulation of the synthesis and secretion of tissue inhibitors of proteolytic enzymes (Dean, 1991).

Although it remains to be established whether changes observed in cartilage metabolism over short-term in vitro cultures would also occur in vivo as a result of long-term administration, the present study suggests that, in contrast to aspirin, tenoxicam at the concentrations found in synovial fluid exerts a favourable effect on the overall metabolism of PG and HA in cartilage with advanced OA lesions. Accordingly, tenoxicam should not per se accelerate joint failure in OA. Tenoxicam, however, did not normalize the changes in cartilage metabolism observed in the OA tissue.

We thank Mr Marchand for his excellent secretarial work. This work was supported by the FRSM grant 3.4553.90 and the Belgian State Prime Minister’s Office, Science Policy Programming (interuniversity attraction poles, grant 7Bis, and concerted actions, grant 89/93.122).

References


(Received December 22, 1993
Revised May 5, 1994
Accepted June 17, 1994)