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J. T. Dingle

The effects of NSAID on the matrix of human articular cartilages

Der Einfluß nichtsteroidaler Antirheumatika auf die Matrix des menschlichen Knorpelgelenkes

Summary The present paper presents data obtained over a 12 year period, on the matrix synthesis and turnover in some 650 arthritic and 180 non-arthritic (N) human cartilages using a standardised in vitro method. When the relative metabolic (synthetic/repair activity) of these human cartilages was compared, it was demonstrated that in osteoarthritis (OA) and rheumatoid arthritis (RA) cartilages synthetic activity was diminished by approximately 50% as compared with N cartilages. However, the turnover rate of matrix was not significantly different between Non-arthritic and OA, but was very substantially increased in RA cartilages compatible with the activity of inflammatory cells and proteolytic enzymes released from pannus. The action of 13 NSAIDs was compared in terms of their effect on cartilage GAG synthesis. 3 of these NSAIDs were also studied in terms of their effect on cartilage collagen synthesis. Consideration of the results in this study and from published material, led to the suggestion that NSAIDs may be divided into 3 categories in respect of their in vitro action on the extracellular matrix of human arthritic cartilages:

1. Those such as Aceclofenac, Tenidap and Tolmetin which can stimulate matrix synthesis
2. Those such as Piroxicam, Tiaprofenic Acid and Aspirin which appear to be without significant effect on matrix synthesis and,
3. Those like Naproxen, Ibuprofen, Indomethacin, Nimezulide which significantly inhibit matrix synthesis.

It is suggested that the stimulatory action of group 1 NSAID is due to inhibition of locally produced IL1 and consequent expression of growth factor activity. Other NSAIDs may also inhibit IL1 synthesis or release, but probably do not have a beneficial effect on chondrocyte synthetic activity as they have toxic effects on cartilage metabolism. These experiments led to the suggestion that NSAIDs such as Aceclofenac would be appropriate for long-term treatment of arthritic conditions provided that one is prepared to extrapolate between in vitro experiments on human cartilage and what may be happening in vivo.

J. T. Dingle (✉)
Addenbrooke's Hospital
Hughes Hall
UK-Cambridge, CB1 2EW

Zusammenfassung Die vorliegende Arbeit präsentiert während einer 12-Jahres-Periode mit einer Standard-in-vitro-Methode gesammelte Daten über Synthese und Umsatz des Gelenkknorpels von 650 arthritischen und 180 nicht-arthritischen (N) Individuen. Beim Vergleich der relativen metabolischen (synthetischen/reparativen Aktivität) dieser menschlichen Gelenkknorpel läßt sich zeigen, daß bei der Arthrose (OA) und der rheumatoiden Arthritis (RA) die Knorpelsynthese um etwa 50% im Vergleich zu N-Knorpeln reduziert ist. Bezüglich des Matrixumsatzes aber gab es keinen signifikanten Unterschied zwischen nicht-arthritischem und arthrotischem Knorpel, während dieser beim RA-Knorpel – vereinbar mit der Aktivität von Entzündungszellen und aus dem Pannus freigesetzten Enzymen – deutlich erhöht war. Die Wirkung von 13 NSAR auf die Glukoseaminoglykansynthese wurde verglichen. Drei dieser NSAR wurden auch auf ihre Wirkung auf die Knorpel-Kollagen-Synthese untersucht. Die Ergebnisse dieser Studie und Literaturangaben führen zu dem Vorschlag die NSAR bezüglich ihrer in-vitro-Wirkung auf die extrazelluläre Matrix des arthritischen Gelenkknorpels in drei Kategorien zu teilen:

1. Substanzen wie Aceclofenac, Tenidap und Tolmetin, die die Matrixsynthese stimulieren können
2. Substanzen wir Piroxicam, Tiaprofensäure und Aspirin ohne si-

gnifikanten Effekt auf die Matrixsynthese und
3. Substanzen wie Naproxen, Ibuprofen, Indometacin, Nimezulide, die die Matrixsynthese signifikant hemmen.

Es ist anzunehmen, daß die stimulierende Wirkung der NSAR der Gruppe 1 auf die Hemmung des lokal produzierten IL-1 und die nachfolgend zunehmende Aktivität von Wachstumsfaktoren zurückzuführen ist. Andere NSAR können Synthese oder Freisetzung von IL-1 ebenfalls hemmen, hemmen möglicherweise aber gleichzeitig aufgrund toxischer Effekte die synthetische Aktivität der Chondrozyten. Diese Experimente weisen darauf hin, daß NSAR wie Aceclofenac für die Langzeitbehandlung der Arthritis geeignet sind, vorausgesetzt, die in-vitro Experimente am menschlichen Knorpel lassen sich auf die Situation in-vivo übertragen.

Key words NSAIDs – cartilage turnover – GAG synthesis – IL-1 inhibition

Schlüsselwörter NSAR-Knorpelumsatz – GAG-Synthese – IL-1-Hemmung

Introduction

Articular cartilage is a highly specialized material consisting of chondrocytes embedded in a two phase extracellular matrix consisting of collagen fibres and glycosaminoglycan (GAG) (1–4). The extracellular GAG is complexed with hyaluronic acid, forming very large aggrecan molecules. The interaction of the collagen and aggrecan allows macro-molecules in the tissue to withstand the recurrent stresses experienced by weight bearing joints (5). This function activity is a property of the 'basket weave' architecture of the collagen matrix which imposes a limitation to the water uptake by the highly charged GAG. The swelling pressure associated with this water content provides an environment which is resistant to compressive forces, as well as providing a low friction surface/surface interaction. The fibrillar collagen, which provides tensile strength, is principally type II, but other minor types including 6, 9, 10 and 11 are also present and have various roles in matrix/matrix and cell/matric interactions. Embedded in this matrix, the indigenous chondrocytes provide a continual synthetic replenishment of the extracellular macro-molecules, the GAG being turned over and re-synthesised considerably more rapidly than the collagen. If the GAG is partially lost from cartilage matrix, the resistance to physical forces is diminished and the articular surfaces becomes susceptible to mechanical damage. The continual metabolic activity of the chondrocyte is essential for the maintenance of the balance between synthesis and degradation which continues throughout life and is largely determined by the interaction of growth factors and cytokines (6, 7). Patients as old as 90 years have been demonstrated to have chondrocyte synthetic capabilities between one third and one half of that from juvenile patients' cartilages. If the integrity of the cartilage is to be maintained, the newly synthesised GAG, collagen and hyaluronic acid must equal the amount lost through normal turnover (8). This turnover is principally due to the activity of catabolic proteolytic enzmyes, particularly the metalloproteinases (9), which in normal tissues are probably produced locally by the indigenous chondrocytes and act within the pericellular environment. Under pathological conditions the activity of these enzymes may be increased from the chondrocytes and also released by inflammatory cells and proliferating synovial cells. In such pathological situations insufficient matrix synthetic activity may fail to replace the components of the matrix lost by enzyme action. It is also important that any drug therapy does not adversely modulate the equilibrium between synthesis and degradation (1, 2).

The present study investigates the action of a variety of non steroidal agents (NSAIDs) that are currently used in the treatment of arthritic conditions (10) and determines their effect on the synthesis of the two major components of articular cartilage matrix namely, GAG and collagen, in (N), (OA) and (RA) tissues (11, 12). Since there is currently no noninvasive quantitative method for measuring human articular cartilage matrix synthetic activity, use has been made of a short-term organ culture technique for articular cartilage (13). This was standardised some 12 years ago and the results presented in this paper are based on measurements on 185 N, 486 OA and 174 RA patients' cartilages which give an estimate of the relative synthetic activity of the ex-vivo material.

Methods

The in vitro methods investigation of GAG and collagen synthetic activity were developed from the original Strangeways Laboratory organ culture techniques for embryonic chick cartilages. These methods were modified for adult cartilages and subsequently standardised for human cartilage. Arthritic and Non-arthritic cartilages were obtained from human femoral heads at operation, the (N) cartilages came from trauma cases. After removal at operation the patient's tissue was immediately transported to the laboratory where samples were taken for histological confirmation of the disease state, the remainder was cut into 4 mm squares of full thickness material. The tissue was cultured, usually for

6 days in 200 µl of DMEM containing 5% foetal calf serum (FCS). The medium was changed on day 3. The gas atmosphere was 5% CO_2, in air. 8–10 replicates were normally set up for each experimental condition. Some 60–70 replicates were usually obtained from each patients' cartilage. GAG synthesis was measured by the incorporation of $^{35}SO_4$ into cetyl pyridinium chloride (CPC) precipital material after a 20 hour pulse with 5 µCi/ml $^{35}SO_4$, at the fifth day of culture. Collagen synthesis measured by the incorporation of ^{3}H-proline into TCA precipital material after 16 days culture in the presence of 20 µCi/ml ^{3}-H proline. Results were expressed on a dry wt or DNA basis and analyzed statistically using Student's t-test. NSAIDs were added at concentrations expected to be found in plasma during treatment (10).

The disease severity of the osteoarthritic cartilage was graded histologically:

1. Slight OA showed local areas of erosion and some loss of metrachromasia.
2. Moderate OA was characterised as having significant fibrillation of the articular surface, loss of metrachromasia around individual cells and some evidence of structural damage.
3. Severe OA showed substantial fibrillation and production of clefts. Substantial to complete loss of metrachromasia and evidence of cellular cloning.

The RA tissues were graded similarly, except in addition, the presence of pannus or loss of cells was also recorded.

Results

1. The metabolism of human cartilage

Table 1 shows the comparison of the cartilage matrix synthesis and turnover in a population of N, OA and RA patients. The data shown is an update on previously published material, with an increase of some 200 OAs, 60 RAs and 100 N cartilages included. In spite of the increased numbers, the actual figures remain similar to that previously published. The OA and RA cartilages have similar GAG synthetic activity which was about half that of the N cartilages. The collagen synthetic activity which had only previously been published for OA cartilages was substantially lower in OA than in RA, which in turn was significantly lower than that in N cartilages. The GAG turnover i.e. the rate at which the GAG is being degraded by the indigenous chondrocytes was similar in the N and OA cartilages but was much higher in the RA patients' cartilages. In particular in those RA cartilages where pannus was present, the turnover rate was raised to 6.5% per day, roughly 4 times that of the Non-arthritic cartilages. When the results are calculated on a DNA basis, which compensates for cell numbers, it is found that this change is metabolism is not due to loss of cells, but is a change in chondrocyte metabolic activity. It should be emphasised that these figures are relative and similar figures would not necessarily be obtained under different conditions.

Table 1 Comparison of matrix synthesis and turnover in a population of non-arthritic, OA and RA patients' femoral head cartilages

	Non-arthritic	OA	RA
Total No. Patients' Cartilages	185	486	174
Mean age [range]	64 (20–87)	68 (48–91)	65 (46–83)
GAG synthesis cpm/mg·10^{-3}	3.64 (0.30)	1.88 (0.38)	1.74 (0.28)
GAG turnover % day	1.60 (0.41)	2.00 (0.40)	3.15 (0.26) [6.5% +pannus]
Collagen Synthesis dpm/mg/16 d·10^{-3} [% Protein synthesis]	2.40 (0.20) [14% (0.6)]	1.00 (0.07) [6.5% (1.0)]	1.71 (0.12) [13% (1.4)]

(SEM)

2. Studies of the effect of NSAID on GAG synthesis in N, OA and RA patients' cartilages

The current studies and other published work (1, 2, 11–19) on the effect of NSAID on human cartilage matrix synthesis are summarised in Table 2. This table includes unpublished work on the effect of Nabumetone, Nimezulide and Paracetamol and also some studies on RA patients' cartilages with Aceclofenac, Diclofenac and Indomethacin.

Considering first the effect of NSAID on N cartilages. It may be seen from Table 2 that Ketoprofen stimulated GAG synthesis in 8 young cartilages but not in 13 adult cartilages. Piroxicam also gave in increase in sulphate incorporation in 10 young cartilages but not in 12 cartilages from older patients. Ibuprofen, Indomethacin, Naproxen, Nabumetone and Nimezulide all showed inhibition of GAG synthesis of adult patients' cartilages which was statistically significant. Aspirin showed significant inhibition in one study of 5 patients' cartilages but not in 2 others involving 20 patients' cartilages. Of the 11 drugs tested, Naproxen appeared to be the most inhibitory on these Non-arthritic cartilages.

Considering next the effect of NSAIDs on OA cartilages; Aceclofenac (60), Tenidap (40) and Tolmetin (6) showed significant stimulation of GAG synthetic activity, whilst Ibuprofen (34), Indomethacin (32), Aspirin (32), Naproxen (64) and Nimezulide (60), all showed

substantial and significant inhibition of GAG synthetic activity. At the concentrations tested TPA (6), Diclofenac (60), Piroxicam (42), Nabumetone (60) and Paracetamol (60) were without significant effect on the population, though some individuals showed significant inhibition with Diclofenac.

In the RA cartilages only Aceclofenac, Diclofenac and Indomethacin have been studied. Aceclofenac again showed a significant stimulation in 60 patients' cartilages, Diclofenac (60) showed a significant inhibition at the same concentration and Indomethacin also showed significant inhibition in 9 patients' cartilages.

Three of these compounds, Aceclofenac, Diclofenac and Naproxen have also been studied in terms of their effect on collagen synthesis in N, OA and RA cartilages. From Table 3 it can be seen that Aceclofenac had no significant effect in 60 Non-arthritic catilages but at the concentrations studied, it showed a significant effect in stimulating collagen synthesis in 40 OA patients' cartilages and in 60 RA patients' cartilages. Diclofenac on the other hand showed a small but significant inhibition on N cartilage collagen synthesis and a significant inhibition at the higher concentrations with OA cartilages (40). In 60 RA cartilages Diclofenac showed a significant inhibition of collagen synthesis at both 5 and 10 μg/ml. Naproxen was highly inhibitory on the N human cartilages and at 100 μg/ml and above was also very inhibitory in OA cartilage. In RA cartilages it was inhibitory to a highly significant degree at both 10 and 100 μg/ml.

Discussion

It has been suggested, on the basis of measurements of GAG synthesis, that NSAIDs tested could be divided into three categories in respect of their effect on OA human cartilage (1, 2). These three categories are as follows:

1. Those agents which increase matrix synthetic activity;
2. Those agents which appear to have little effect on chondrocyte metabolic activity except at higher concentrations and,
3. Those agents which are substantially inhibitory of cartilage metabolism and hence may decrease the repair potentiality of cartilage.

The present studies have looked at further NSAIDs and also the action of examples of each of these categories on RA cartilage and also on collagen synthesis. The results in general confirm the previous observations and the hypothesis arising from these observations. Aceclofenac appears to be consistently stimulatory in terms of both GAG and collagen synthesis on arthritic cartilages but not on Non-arthritic cartilages. Diclofenac, at higher concentrations, is significantly inhibitory on Non-arthritic cartilages and Rheumatoid arthritic cartilages but at low concentrations it is not inhibitory on OA cartilage. At higher concentrations, although they are not shown in the present Tables, it is inhibitory. Diclofenac was significantly inhibitory to collagen synthesis on N, OA and RA cartilages. Naproxen was substantially and significantly inhibitory in all categories of cartilages investigated. Nimezulide was shown to be inhibitory on both Non-arthritic and OA cartilages but not tested on RA material whilst Nabumetone was inhibitory on Non-arthritic was without significant effect in the OA material. Paracetamol which was investigated as a control in some 60 OA patients' cartilages, had no discernable effect on GAG synthesis.

Whilst the inhibitory action of some of these NSAIDs can be explained by cytotoxic effects, and in particular the difference between Diclofenac and Aceclofenac is explicable in terms of toxic metabolite production (1, 20), it has been hard to understand the stimulatory nature of some of these agents, since one would not normally associate them with growth factor like activity. However, recent work has enabled a hypothesis to be developed for the action of Aceclofenac and other stimulatory NSAIDs (21).

The action of Aceclofenac is suppressing IL1 activity has been demonstrated previously in an ex-vivo study by Gonzalez et al. (22). These authors showed diminished IL1 and TNF activity after treatment with Aceclofenac (100 mg twice daily) in patients' whose basic cytokines levels were high. Since we know that IL1 is extremely active in suppressing GAG and collagen synthesis (23) in human articular cartilages, any therapeutic agent which modifies local IL1 activity might be expected to influence the activity of indigenous chondrocytes. It has been demonstrated that this occurs in arthritic tissues provided that the growth factor activity in present (21). For example, IGF1 which stimulates human chondrocyte matrix synthetic activity, will be expressed, if indigenous IL1 levels are reduced. In OA (24) the catabolic reactions will be sufficient over a period of months to cause sufficient local loss of GAG and collagen if growth factor activity is suppressed by IL1 (1). In RA is it probable that the great sensitivity to IL1 of matrix synthesis coupled to the effect of active proteolytic enzymes associated with pannus will be responsible for the more rapid loss of matrix seen in this disease (2) and also demonstrated in the in vivo studies (25). A number of NSAIDs probably are capable of diminishing IL1 activity but in order to demonstrate increased matrix synthetic activity potential, these agents must also be demonstrated to be non-cytotoxic since there is a balance between diminishing cytokine activity and allowing growth factor mediated repair mechanisms to take place. Aceclofenac at the

concentrations tested, which include the highest concentrations which are present in human plasma during treatment, did not show cytotoxic effects and did allow an increased matrix synthetic activity in the presence of growth factors. One must, of course, exercise caution in extrapolating from these in vitro experiments to in vivo situation in the human patient. There is evidence from studies on patients treated with Indomethacin by Rashad et al. (26) and Huskinsson et al. (27) that Indomethacin has a deleterious effect on cartilage. A finding which is compatible with the studies reported in this paper. It is possible, therefore, that the modification of the cytokine/growth factor interactions in arthritic cartilages which has been demonstrated in these short-term in vitro studies may occur in in vivo during treatment with agents such as Aceclofenac and could lead to the stimulation of matrix synthesis and possible repair.

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