Joint capsule collagen in osteoarthritis

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It has long been recognized that although osteoarthritis is characterized by degenerative changes in the articular cartilage and bones, changes in the capsule play an important part in the symptoms. The capsule becomes thickened and lacks its normal pliability, thus restricting normal movement (Gade, 1947; Apley, 1969) and resulting in pain in the joint. Similarly, Jayson (1969) described a series of patients with arthralgia and increased joint capsule stiffness. Incision, or partial removal of the osteoarthritic capsule, relieves the pain and improves the range of movement and degree of deformity (Lloyd-Roberts, 1953; Gade, 1947). The joint cavity and articular surfaces have no pain receptors whilst the capsule is richly supplied with pain fibres (Gardner, 1948; Kellgren and Samuel, 1950), strongly suggesting that the stiffening of the capsule leads to pressure on the pain receptors (Apley, 1969). The mechanical stiffness of the capsule depends to a large extent on the fibrous protein collagen. Changes in the quantity

and stability of the capsular collagen could lead to the observed symptoms.

Recent studies have established that the stability of the collagen fibre depends upon the formation of inter-molecular cross-links between the tropocollagen molecules making up the fibre (Bailey, 1968; Traub and Piez, 1971). These cross-links are formed extracellularly through reaction of aldehydes derived from specific lysine and hydroxylysine on the collagen molecule (see Scheme).

There is now a well-documented pattern of evolution of these inter-molecular cross-links. The links initially formed are labile Schiff-bases and may be stabilized and detected by reduction with tritiated borohydride. Using this technique they have now been characterized in normal skin, tendon, bone, and cartilage collagen (Fowler and Bailey, 1972), each tissue revealing a unique distribution of these reducible cross-links. These labile bonds are, however, only intermediates and are found in highest concen-

Scheme Formation of cross-links

trations during the rapid growth period and have virtually disappeared by maturity (Bailey and Shimokomaki, 1972) to form stable non-reducible cross-links, the structure of which has not yet been elucidated. This pattern has been established with each of the above tissues and it seemed likely that, if a similar pattern occurred in joint capsule collagen, it would be possible to detect any abnormalities in the collagen cross-linking in osteoarthritis. This paper reports that, in osteoarthritic joints, the changes in the capsules are due to the laying down of new collagen rather than to degeneration or alteration of the collagen already present, suggesting that it may be possible to cleave these new bonds and relieve the pain.

Material and methods

Specimens of hip joint capsule were obtained from fifteen cadavers within 24 hours of death (age range 0 to 75 yrs). Gross observation, clinical notes, x-rays, and a study of the dissected specimens satisfied us that the joints were normal.

Capsules from osteoarthritic joints (12) were specimens obtained at surgery for osteoarthritis of the hip in adult subjects (age range 30 to 70 yrs). The material was always obtained from the same location in the joint. The hip capsule was chosen because osteoarthritis in this joint frequently demands operation.

Articular cartilage was dissected from the unaffected areas of the surface of the head of the femur from osteoarthritic subjects.

Preparation of intact collagen fibres

The capsule was cleaned of adhering fat and muscular tissue and shredded in an M.S.E. Ato-Mix homogenizer and washed with copious amounts of isotonic saline (0.9 per cent NaCl; pH 7.4) to remove soluble protein and glycosaminoglycan. Hydroxyproline determinations showed the tissue to be over 90 per cent collagen.

Reduction of collagen fibres

Samples (250 mg. freeze-dried tissue) of the intact collagen fibres were suspended in 0.9 per cent NaCl (pH 7.4) and reduced with tritiated potassium borohydride (KB^3H_4) diluted with non-radioactive KBH_4 to 10 mCi/mmol. (30:1 weight ratio collagen: KBH_4). For direct comparison of tissue of different ages, all samples were reduced concurrently, the KB^3H_4 being dissolved in the saline (0°C) and equal portions of the solutions being used for the reduction. The reaction was allowed to proceed for 1 hr, after which time acetic acid was added to a final pH 4 and the mixture dialysed against distilled water overnight, freeze-dried, and weighed.

Identification of the cross-links

The weighed samples were hydrolysed in boiling 6N HCl for 24 hr. The HCl was removed by evaporation *in vacuo*, and the hydrolysate then analysed by ion-exchange chromatography, using the Technicon analyser with pyridine-formate buffers (Bailey, Peach, and Fowler,

1970). All the eluent from the column was collected in 5-ml. fractions, using an automatic fraction collector. Each fraction was then analysed for tritium activity by taking aliquot (0.2 ml.), adding Bray's (1960) solution (3 ml.), and counting in a Packard Scintillation Counter. The identity of the radioactive fractions was confirmed by analysis of a portion on the Beckman Amino Acid Analyser, using an extended (60 cm.) basic column. The normal amino acids were located by paper chromatography of the fractions against known standards.

Treatment with D-penicillamine

Samples of extensively washed osteoarthritic capsules were immersed in a solution of 0.1 or 0.001 M D-penicillamine (0.9 per cent NaCl; pH 7.4) for 48 hours at 38° C. Osteoarthritic capsules incubated under similar conditions, but in the absence of D-penicillamine, acted as controls. To prevent bacterial contamination, the solutions were covered in a layer of toluene. Both the treated and control samples were then analysed for reducible cross-links and solubility changes.

(a) REDUCIBLE CROSS-LINKS

The treated and control samples were washed in distilled water and reduced with potassium borohydride as described above and the effect on the reducible cross-links recorded.

(b) SOLUBILITY

The treated and control samples were dialysed overnight to remove salt and the insoluble residue which remained was freeze-dried and weighed.

Results

Changes with age in the reducible cross-links of capsule (Fig. 1, overleaf)

The chromatographic elution pattern of the radioactive components obtained from borotritide reduced joint capsule (Fig. 1a) was found to be very similar to that previously obtained for tendon. The major reducible cross-links were shown to be present in joint capsule, dehydro-dihydroxylysino-norleucine (dehydro-diOH-LNL), and dehydrohydroxylysino-norleucine (dehydro-OH-LNL). A smaller but significant proportion of an additional cross-link present was the as yet uncharacterized Fraction C. With increasing age of the capsule, the proportion of these Schiff-base cross-links was found to decrease, whilst the lysyl carbohydrate components (Fraction A) increased until, at about 20 to 25 years of age, the Schiff-bases had virtually disappeared (Fig. 1b).

Analysis of reducible cross-links in osteoarthritic capsule collagen

Capsules obtained from patients covering the age range 30 to 70 yrs were found (Fig. 1c) to possess a high proportion of the two reduced cross-links, diOH-LNL and OH-LNL previously shown to be absent in all normal subjects over the age of 20 yrs.

FIG. 1 Typical distribution of radioactive components obtained from an acid hydrolysate of joint capsule collagen reduced with tritiated borohydride and separated on a Technicon Amino Acid Analyser. (a) Normal joint capsule from subject 8 yrs old. (b) Normal joint capsule collagen from subject over 30 yrs old. (c) Osteoarthritic joint capsule collagen from subject over 30 yrs old. Positions of reducible intermolecular crosslinks B1, B2, and C shown in relation to the usual amino acids.

All osteoarthritic joints so far examined have exhibited this unusual pattern. Obviously the proportion of these is dependent on the age at onset of the condition rather than age when examined; hence the values are depicted in Fig. 2 as independent points together with the curve showing the variation in normal capsules.

Analysis of reducible cross-links in osteoarthritic cartilage collagen

Articular cartilage collagen possesses a slightly different pattern from joint capsule collagen, dihydrodiOH-LNL being the only major reducible cross-link (Fig. 3a). As in the case of the capsule collagen, the proportion of this reducible cross-link decreased with age (Fig. 3b). Analysis of the fibrillated cartilage remaining in specimens of osteoarthritic joints all produced the same cross-link pattern as the normal subjects (Fig. 3b).

FIG. 2 Variation in production of reducible intermolecular cross-links (B1 + B2) in relation to the total amount of collagen with increasing age in normal subjects (●—●) and osteoarthritic subjects (▲).

FIG. 3 (a) Elution pattern of an acid hydrolysate of tritiated borohydride-reduced articular cartilage collagen of subject less than 20 yrs old. (b) Variation in proportion of major reducible cross-link (B1) with increasing age from normal cartilage (●—●) and osteoarthritic joints (▲).

Effect of D-penicillamine on osteoarthritic capsule collagen

(A) REDUCIBLE CROSS-LINKS

Cleavage of the labile Schiff base cross-links by the D-penicillamine was found to occur by reduction of the fibres after this treatment and noting the decrease in amounts of the cross-links (Fig. 4a). The effectiveness of the treatment varied somewhat but in all cases a significant decrease occurred (Fig. 4b).

FIG. 4 (a) Effect of D-penicillamine on the elution pattern of the reducible components present in osteoarthritic joint collagen. Note marked decrease in proportion of B1 and B2. Osteoarthritic capsule collagen (—) and D-penicillamine-treated osteoarthritic capsule collagen (---). (b) Variation in amounts of B1 + B2 in osteoarthritic capsules before and after treatment with D-penicillamine.

(B) SOLUBILITY

The increase in the amount of collagen extractable by D-penicillamine due to cleavage of some of the labile cross-links is shown in Fig. 5. 0.1 M D-penicillamine was found to be much more effective than 0.01 M, but again considerable variation in the efficacy of the penicillamine treatment was observed.

Discussion

After having established that the major reducible cross-links of joint capsule collagen were present only

FIG. 5 Effect of D-penicillamine on solubility of joint capsule collagen from osteoarthritic subjects of various ages.

in minor quantities after the age of about 20 years, analysis of osteoarthritic subjects in the age range 30 to 70 years all revealed a high proportion of these labile intermediate cross-links. No analogous change in the articular cartilage collagen was observed. The results obtained clearly show that, in contrast to the degenerative changes occurring in the articular surfaces, the changes taking place in the joint capsule are partly due to the laying down of new collagen rather than to the alteration of the collagen already present.

Unfortunately, generalized fibrosis of the capsule seems to occur and in advanced cases the synovial membrane becomes almost obliterated. Consequently, it was not possible to study the effect on the membrane independently as was possible with the grossly thickened membrane in rheumatoid arthritis (Herbert—unpublished). This generalized proliferation of collagen in the capsule may be due to a compensatory action for the loss of the smooth working action of the joint surfaces, and in response to the irritating affect of fragments of bone and cartilage dislodged from the fibrillating articular surfaces (Lloyd-Roberts, 1953). It is possible that the thickening and reduction in range of movement of the capsule could have an important influence on the progress of the degeneration of the joint, since it would result in a progressive decrease in the contact area of the load-bearing articular surfaces, thus leading to increased degeneration of the cartilage. It is clear, therefore, that any treatment producing a reduction in the tension of the capsule may have the effect of both relieving pain and of decelerating the degeneration of the joint surfaces. The ability of one compound, D-penicillamine, to cleave some of the cross-links *in vitro* and consequently to soften the

capsule has been demonstrated, and could provide such relief. Further studies of the application of this approach *in vivo* are indicated.

Summary

Analysis of joint capsule collagen from the hips of osteoarthritic patients revealed the presence of a high proportion of the intermediate Schiff-base cross-links normally present only in immature subjects. The presence of these cross-links indicate the laying down of new collagen in the capsule rather than degeneration of collagen already present. Since the symptoms of osteoarthrosis arise partly through the thickening and stiffening of the joint capsule, cleavage of the labile cross-link of this newly-formed collagen may soften the capsule and relieve the symptoms. D-penicillamine has been shown to be effective in cleaving the bonds *in vitro*, indicating that studies *in vivo* could be of value.

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Discussion

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Do any other substances such as guanidine or urea cleave in the same way as penicillamine? May I caution against extrapolating from this phenomenon of the biochemical laboratory to a clinical context either in therapy or in symptomatology.

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Yes, other substances will cleave; cysteamine acts in a similar way to penicillamine. Guanidine and urea, however, are well known denaturing agents. Obviously the system is laboratory orientated, but we hope to extend these findings to studies *in vivo*.