Clearance of proteoglycan from joint cavities

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SUMMARY The rate of loss from the synovial cavity of proteoglycan subunit, a major constituent of cartilage, has been measured in rabbits with and without experimental arthritis. The effect of aggregation between proteoglycan and hyaluronic acid upon the rate of elimination has also been investigated. The results indicate that proteoglycan subunit has a clearance half life of around 12 hours in normal joints and that this rate is not significantly altered in the presence of an active synovitis. Neither injection of proteoglycan as an aggregate, nor in a form incapable of aggregation, had any significant effect on clearance rates. These data indicate that loss of proteoglycan from the joint is not retarded by its ability to aggregate with hyaluronic acid in the synovial fluid and, together with measurements of synovial fluid proteoglycan, may enable rates of cartilage breakdown in vivo to be calculated.

Key words: experimental arthritis, rabbits, articular cartilage, glycosaminoglycans, metabolism synovial fluid, synovium.

The characteristic joint lesions in osteoarthritis (OA) and rheumatoid arthritis (RA) are associated with loss of proteoglycan and collagen from the articular cartilage. Recent work has indicated that measurement of proteoglycan in synovial fluid may be a relatively simple and reliable means of determining the rate of loss of proteoglycan during the disease process. Saxne *et al* in Lund using immunochemical methods¹ and G Carroll at the Strangeways Laboratory using a chemical method (personal communication) (which correlated well with immunochemical assays) demonstrated the presence of substantial quantities of glycosaminoglycans (GAGs) in synovial fluids. Dingle *et al* have assayed GAG levels in over 150 OA and RA fluids.² The chemical methods involve a modification of the dimethylmethylene blue assay technique for proteoglycans.³ This method can also be applied to animal synovial fluids. Dingle *et al* showed that the treatment of rabbit knee joints with a synovial interleukin 1 preparation caused a loss of articular cartilage proteoglycan and a concomitant rise of GAGs in the synovial fluid.⁴ To establish the quantitative relation between the level of GAGs in synovial fluid and the rate of loss from the articular cartilage it is necessary to determine the clearance rate of proteoglycan from the synovial fluid. The

work described in this paper measures the clearance rate of aggregate, subunit, and non-aggregated subunit from rabbit knee joints.

Materials and methods

The proteoglycan was prepared from bovine nasal septa by the method of Heinegård,⁵ and part of the subunit was rendered non-aggregatable by acetylation.⁶ Proteoglycan aggregate, subunit, non-aggregatable subunit, and bovine serum albumin (BSA) (Sigma Chemical Co, Poole, Dorset) were labelled with ¹³¹I using 'Iodobeads' (Pierce Chemical Co, Rockford, Illinois).⁷ ¹³¹I (specific activity 500 MBq/μg) (Amersham International, Aylesbury Bucks) was added to a solution of the appropriate protein (1 mg/ml) in phosphate buffered saline, pH 7.35, to give a final radioactive concentration of 37 MBq/ml. One Iodobead was added for each ml of solution and the reaction mixture was allowed to stand with occasional agitation for 10 minutes. The Iodobeads were then removed and carrier sodium iodide added to give a final concentration of 100 mg/ml. This solution was dialysed for a total of 72 h in two changes of deionised water and a third of physiological saline. All the above procedures were carried out at 4°C. Labelling by this method yielded products with specific activities of between 4 and 10 MBq/mg protein. The subunit, aggregate, and acetylated subunit were characterised on a Sepharose 2BCL column and did not appear to

Accepted for publication 31 May 1987.
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suffer any change in molecular size or in ability to aggregate after iodination.

ANIMAL EXPERIMENTS

Rabbits were of the Old English strain and between 3 and 9 months old. Unilateral experimental arthritis⁸ was induced in the knees of some of these animals 21 days before the following experiments were carried out.

The rabbits were sedated with 'Hypnorm' (0.4 ml/kg) (Janssen Pharmaceuticals, Beerse, Belgium) and 0.4 ml of a radiolabelled protein solution injected into the synovial cavities of their knees. The rabbits were positioned rapidly with their knees underneath vertical, lead collimated, gamma scintillation probes and the radioactivity measured as previously described.⁹ Counting was repeated at

Fig. 1 Loss of proteoglycan subunit preparations from normal and experimentally arthritic rabbit knees: ○—○ Pg=proteoglycan, normal knees; ●—● Pg(A)=proteoglycan, arthritic knees; □—□ NA=non-aggregatable subunit, normal knees; ◇—◇ ISU=inhibited (acetylated) subunit, normal knee. Points represent pooled results for each time point and the lines are least mean squares fits to the respective data.

Table 1 Clearance rate of proteoglycan preparations in normal and arthritic rabbit knees

Preparation	Normal knees		Arthritic knees	
	Half life (h)*	n	Half life (h)	n
Proteoglycan aggregate	12.94 (0.63)	14	14.49 (2.58)	5
Proteolytic proteoglycan subunit	12.47 (0.68)	4	ND†	
Non-aggregatable subunit	12.47 (0.34)	5	ND	
BSA†	3.91 (0.81)	3	ND	
Sodium pertechnetate	0.82	1	1.78	1

*Values are mean (SEM).
†BSA=bovine serum albumin; ND=not determined.

intervals for periods of up to 48 h, and in some experiments the animals were then killed by means of an intravenous injection of 'Euthatal' (May & Baker Ltd, Dagenham, Essex). The joints of these animals were opened, the synovial fluid collected, the synovial cavity washed twice with 0.25 ml physiological saline, and the synovial fluid combined with the washings. The synovium was also removed. The tissues and the joint washings were weighed and their radioactivities counted on a Packard Multi-Prias 4 gammacounter using a window of 15–500 keV. All radioactive measurements were corrected for the decay of ¹³¹I (half life 8.07 days).

Results

Radiation measurements over the knees declined exponentially with time (Fig. 1) ($r>0.9$) with all of the preparations injected. It was thus possible to calculate the clearance rate from the knee in each case as a half life, and these values are summarised in Table 1. All the proteoglycan preparations had clearance half lives of between 12 and 13 hours in normal (non-arthritic) joints, and although the mean value for total proteoglycan subunit was slightly higher than this in arthritic joints, the difference was not significant (Table 1). Removal of the aggregatable fraction from the injected material, or rendering the proteoglycan non-aggregatable by acetylation had no effect on its clearance from joints. Proteoglycan was, however, released significantly more slowly than BSA (Table 1; $p<0.001$).

Measurements of the residual radioactivity in the synovium and the synovial cavity showed that at 42 h after injection of proteoglycan subunit into normal joints a total of 1.63 (SEM 0.23)% of the injected radioactivity could be recovered from the joint, with approximately half in the synovial fluid and the remainder in the synovium (Table 2). The total radioactivity recovered and its distribution between synovial fluid and the synovium were not signifi-

Table 2 Recovery of radioactivity from the synovium and synovial fluid 42 h after intra-articular injection of proteoglycan subunit preparations

Preparation	Synovial fluid	Synovium	Total
Subunit (normal knee) (n=9)	0.89 (0.21)†	0.76 (0.09)	1.63 (0.23)
Subunit (arthritic knee) (n=5)	1.48 (0.46)	1.57 (0.46)	3.06 (1.14)
Non-aggregatable subunit (normal knee) (n=4)	0.22 (0.11)	0.57 (0.04)	0.79 (0.13)*

* $p < 0.05$.

†Values are mean (SEM) and given as a percentage of the total radioactivity injected.

cantly different in experimentally arthritic joints. Less radioactivity remained, however, in normal joints injected with non-aggregatable material ($p < 0.05$) (Table 2).

Discussion

Saxne *et al* have shown using immunoassay techniques that substantial quantities of proteoglycan are released into the synovial fluids of patients with various knee joint arthritides.¹ Levels of proteoglycan antigen of the order of 5–10 mg/joint were reported. In a preliminary communication Dingle *et al* have used chemical assays for glycosaminoglycans to determine GAG levels in some 150 synovial fluids from arthritic patients.² Mean values of 200 µg/ml (range 45–500 µg/ml) were found in a population of rheumatoid patients and 430 µg/ml (range 300–800 µg/ml) in the synovial fluid of patients with osteoarthritis. When the volume of the synovial fluids is taken into account this represents approximately 5 mg (range 1–20 mg) of GAG in joint fluid in the whole knee in the rheumatoid patients and approximately 2 mg (range 1–2.8 mg) GAG in the joint fluid of the osteoarthritic patients—values which are similar to those obtained by Saxne *et al*.

When the rate at which the cartilage matrix may be lost during the course of the arthritic disease is considered these high values appear to point to a very rapid catabolic activity, bearing in mind that the intact adult human knee joint contains about 1–1.5 g GAG (Dr Baylis, Kennedy Institute, personal communication). To determine the application of these values to the turnover of the cartilage matrix it is necessary to have an estimate of the clearance rate of the GAGs from the synovial fluid. As it was unethical to carry out the necessary

procedures on humans we decided to use normal and experimentally arthritic rabbits.

We used the Page-Thomas model for rabbit arthritis⁸ as this gives a reproducible and well characterised lesion with a known rate of development. Rabbit joints are, furthermore, large enough to monitor reproducibly with external gamma scintillation probes.

The results obtained show that proteoglycan is cleared from the rabbit knee with a half life of approximately 12 h (Table 1). In contrast, the clearance half lives of albumin (mol. wt 65 000) and sodium pertechnetate (mol. wt 201) were approximately 3.9 h and 0.82 h respectively, even though the pertechnetate anion becomes partially bound to protein in biological fluids.¹⁰

Proteoglycan subunit is a polydisperse macromolecule with a mean molecular weight of around 2.5×10^6 (SD 1.2×10^6)¹¹ and it seemed likely that its relatively slow rate of clearance in comparison with albumin and pertechnetate was attributable to its much higher molecular weight. It might therefore have been expected that native subunit, which has the ability to aggregate with hyaluronate to form yet larger complexes, would be cleared even more slowly than proteoglycan in which this property was impaired. This hypothesis was examined by comparing the clearance rates of proteoglycan subunit, preformed proteoglycan/hyaluronate aggregate, and proteoglycan which had been rendered incapable of aggregation by acetylation. There was no significant difference in the clearance rates of these different preparations, suggesting that the acetylated subunit was sufficiently large for passive diffusion to be minimal and that the bulk of the material was cleared from the joint by other processes.

Small quantities of both aggregatable and non-aggregatable material could be recovered from the synovial fluid and the synovium 42 h after injection. The small, but not significant increase in the retention of aggregatable material in these tissues from joints with experimental arthritis is a reflection of the larger weights of tissue and fluid recovered and does not indicate differences in tissue concentrations. Significantly more radioactivity was, however, present in normal joints injected with aggregatable as opposed to non-aggregatable material ($p < 0.05$), perhaps suggesting a slower tissue breakdown rate for the aggregate.

As no figures are available for the clearance rate of proteoglycan material from human synovial fluid and it is reasonable to assume that similar physiological processing applies in both the rabbit and man, one is perhaps justified in making a very cautious extrapolation from the animal clearance rates reported in this paper to those in the human knee

joint. If the clearance rates of approximately 12 h occurred also in human arthritic patients this would suggest that some patients with RA could be losing >10 mg of GAG/day during the active phase of the disease. Osteoarthritic patients might be losing up to a third of this quantity a day. These figures are calculated from a population of 85 patients with RA (mean 6 mg/joint) and 57 with OA (mean 2.5 mg/joint)² and may be unrepresentative of the activity in any individual patient if this varies with time.

Saxne *et al*, however, showed that two untreated patients sampled at one and three month intervals maintained constant levels of proteoglycan.¹ Unpublished studies by Hazleman, Cawston, Page-Thomas, and Dingle, in which a longitudinal study of secretion of GAGs by individual patients was carried out, have shown also that the level of GAG in synovial fluid can remain high over a period of several months. Although much work remains to be done in this area, these results suggest that consideration of proteoglycan loss into the synovial fluid should be a factor in the assessment of any treatment which aims to maintain the integrity of cartilage.

This work was supported by funds from the Arthritis and Rheumatism Council and the Medical Research Council.

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