Effect of synovial fluid hyaluronan on the clearance of albumin from the canine knee

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The synovial fluid concentration of a variety of macromolecules derived from the matrix of the articular cartilage, including matrix proteins, proteoglycan fragments, and glycosaminoglycans, has been related to the magnitude of cartilage destruction or repair in arthritic joints. One determinant of the concentration of these molecules in the synovial fluid is their rate of release from the cartilage, and they serve as practical molecular 'markers' of disease in studies of osteoarthritis and rheumatoid arthritis. However, marker concentration in synovial fluid specimens also depends on factors that influence the clearance of the molecule from the synovial fluid. These include the volume of fluid within the joint space, synovial blood flow and inflammation, joint motion and, potentially, the composition of the synovial fluid. Unless these factors are taken into account, differences in marker concentration seen in serial samples of synovial fluid may misrepresent the metabolic status of the articular cartilage.

Cartilage derived marker molecules and most proteins, for example albumin, appear to be removed from the joint space at approximately the same rate. Thus when 131I-labelled serum albumin (RISA) is injected into the knee its clearance kinetics approximate those of marker clearance from the joint space. In the present study we examined the relationship between the composition of the joint fluid (the concentration and average molecular weight of the hyaluronan therein) and the kinetics of RISA clearance from the normal knee.

Materials and methods

HYALURONAN IN CANINE SYNOVIAL FLUID

The knees of normal dogs (13–25 kg) were aspirated to obtain 0.05–0.25 ml of synovial fluid, and repeated saline lavage of the joint was used to recover all remaining synovial fluid. The uronic acid concentration and intrinsic viscosity of these specimens were measured to determine the hyaluronan concentration and content of the synovial fluid, and hyaluronan average molecular weight.

MANIPULATION OF SYNOVIAL FLUID HYALURONAN

The right knee, and one week later the left knee, of 13 beagle dogs (13–15 kg) was injected with 0.5 ml of a preparation of saline and 5 μCi RISA that contained 0.03 mg/ml or 3.0 mg/ml of 6 × 10⁶ Da hyaluronan (RISA-HA₆), or 0.3 mg/ml or 3.0 mg/ml of 2 × 10⁶ Da hyaluronan (RISA-HA₂). The table shows the pairing of injections. The resulting concentration of hyaluronan in the synovial fluid was measured (t = 0).

DETERMINATION OF RISA CLEARANCE

After each injection of RISA, hourly measurements of iodine-131 (cpm) were taken over the anterolateral aspect of the joint for seven hours, at which time (t = 7 h) all available synovial fluid was aspirated and its volume and radioactivity (cpm/ml) were determined. The hyaluronan concentration and intrinsic viscosity of representative samples were measured, and the apparent distribution volume (Vd) and clearance (μl/min) of RISA were calculated as described by Simkin.

Results

In specimens of synovial fluid from normal dogs the concentration of hyaluronan averaged 2.9 (SD 0.5) mg/ml, and its average molecular mass was 1.4 × 10⁶ Da. Because joint aspiration has been shown to underestimate the volume of fluid present in the joint, saline lavage was used to determine that 0.5–1.7 mg of hyaluronan could be recovered from the normal canine knee; recovery from right and left knees was similar (figure). A strong correlation was seen between the amount of hyaluronan recovered and body weight.
The concentration of hyaluronan in joint effusions from patients with osteoarthritis and inflammatory arthropathies is less (0.8–1.7 mg/ml) than that in normal synovial fluid (1.5–2.9 mg/ml)\(^8\) and its average molecular mass is also reduced.\(^9\)\(^10\) Theoretically, steric exclusion of synovial fluid proteins, including those derived from the articular cartilage, from those fractions of the synovial fluid and synovial tissue matrix that are occupied by hyaluronan\(^11\)\(^12\) could affect the volume of distribution and clearance of these proteins from the joint.\(^6\) Non-covalent interactions also have been described between hyaluronan, aggrecan, and a variety of other proteins, including albumin.\(^13\) Thus the changes in synovial fluid composition seen in arthritic joints could alter the clearance kinetics and synovial fluid concentration of ‘marker’ molecules.

The results of the present study support this possibility, as a slight but significant decrease in RISA clearance was detected in knees in which the synovial fluid concentration of hyaluronan was reduced to subphysiological values comparable to those seen in human synovial effusions. In this series, changes in the molecular mass of synovial fluid hyaluronan did not have a discernible impact on protein clearance from the normal knee. Because substantial manipulation of synovial fluid hyaluronan in these experiments produced only minor changes in RISA clearance, we conclude that other factors, such as synovitis, joint distension, or involuntary splinting of the painful extremity are probably more significant than hyaluronan as determinants of the kinetics of marker clearance in the arthritic joint.

\(^13\) Laurent T C, Ogston A G. The interaction between poly- saccharides and other macromolecules. 4. The osmotic pressure of mixtures of serum albumin and hyaluronic acid. \textit{Biochem J} 1963; 89: 249–53.