

Hyaluronan in inflammatory joint disease

Torvard C Laurent¹, J Robert E Fraser², Ulla B G Laurent³
and Anna Engström-Laurent⁴

Departments of ¹Medical and Physiological Chemistry, BMC, Box 575, S-751 23 Uppsala, Sweden and ³Ophthalmology, University of Uppsala, and ⁴Rheumatology Clinic, Regional Hospital of Falun, Sweden; ²Laboratory of Fetal and Neonatal Immunology, Faculty of Veterinary Science, Melbourne University, Australia.
Correspondence: T C Laurent. Tel +46-18-17 41 55. Fax +46-18-17 49 75.

Hyaluronan (HYA) (Laurent et al. 1992) is a linear extracellular polysaccharide found in all tissues and body fluids but in highest concentrations in loose connective tissue. It is present in synovial fluid, where it is responsible for the unique rheological properties, in cartilage, where it forms the backbone of the proteoglycan aggregates and in the synovial tissue and joint capsule. HYA is also present in muscular tissues between the muscle bundles.

As joint disease often is accompanied by swollen joints and increased volume of synovial fluid it has been popular to study the changes in composition and rheological properties of the fluid. Most investigators have shown a drop in viscosity and a lower concentration of HYA in arthritic joint fluid (Sundblad 1965, Seppälä 1964, Balazs et al. 1967, Dahl et al. 1985).

When new techniques were introduced, which made possible the analysis of HYA in blood, it was observed that the serum level of HYA often was increased in patients with rheumatic joints. In the present communication we will examine the evidence that the excess HYA in blood originates from the affected joints.

Serum hyaluronan in patients with joint disease

The normal levels of HYA in blood serum were recorded about ten years ago. A middle aged adult has a mean level in the order of 30-40 ng/ml (Engström-Laurent et al. 1985, Lindqvist and Laurent 1992). Pathologically high serum HYA levels have been recorded in patients with liver disease, especially liver cirrhosis; with inflammatory conditions e.g. rheumatoid arthritis (RA); with HYA producing tumors e.g. Wilms' tumor or mesothelioma; or with some rare genetic conditions, e.g. Werner's disease (Engström-Laurent and Laurent 1989). Only joint disease will be discussed below.

The first observations on elevated levels of serum HYA in inflammatory joint disease were made by Engström-Laurent and Hällgren (Engström-Laurent and Hällgren 1985). A number of reports both on patients (e.g. Engström-Laurent and Hällgren 1985, Seibel et al. 1988, Levesque et al. 1988, Poole et al. 1990, Goldberg et al. 1991, Paimela et al. 1991, Andersson Gäre and Fasth 1994) and animals with experimental arthritis (e.g. Björk et al. 1989) have confirmed these results.

The steady-state level of HYA in blood is determined by the input of the polysaccharide into blood from the tissues, which was shown to be via the lymph (Laurent and Laurent 1981) and the clearance of circulating HYA, which is taking place mainly in the liver endothelial cells (Fraser et al. 1981, Smedsröd et al. 1984). The clearance of HYA has been measured in normal persons and patients with RA. There is no impairment in the clearance in the patients, rather the opposite (Fraser et al. 1986, Lindqvist et al. 1992).

Engström-Laurent and Hällgren (1987) collected blood from patients with RA both when the subjects woke up in bed in the morning and one hour after getting out of bed. The HYA level in serum rose dramatically during the first hour of normal physical activity in the morning. Similarly, physical exercise in the afternoon raised the level. The effect of exercise on the HYA level of normal individuals was much less accentuated. A reasonable explanation would be that the physical activity pumps lymph with high amounts of HYA from the rheumatoid joints into the circulation of the patients. This conclusion was strengthened by the observation that the elevation of serum HYA during exercise correlated with the duration of morning stiffness in the joints of the patients. The authors concluded that HYA presumably accumulates in the joints during rest, causing edema and stiffness, and is

removed by physical exercise. The variation of serum HYA with physical activity has subsequently been confirmed by other authors (Lindqvist et al. 1988, Chassagne et al. 1989, Saari et al. 1991). The wash out of HYA into the circulation of rheumatoid patients seems to be correlated to the number of joints afflicted (synovitis mass) (Engström-Laurent and Hällgren 1987, Poole et al. 1990, Paimela et al. 1991).

The rapid variations in the level of serum HYA in one and the same patient makes it difficult to use analytical routine analyses for following the extent and progression of RA. However, the observations of elevated levels in patients with RA have afforded us with new important information on the role and behavior of this polysaccharide in joint disease and it is quite possible that future refined analyses, e.g. continuous recording with a biosensor, will yield more reliable clinical information. To determine daily urinary excretion of hyaluronan is another possibility, which has not yet been fully explored. It has been reported that the excretion is doubled in patients with RA (Laurent et al. 1987).

Hyaluronan from joints is transported to blood

The first evidence that HYA can be transported from the joints by lymph to blood was obtained by Antonas et al. (1973) who injected ^{14}C labeled HYA into rabbit knee joints and recovered radioactivity in cartilage, synovial tissue, regional lymph nodes and blood. It has later been shown that lymph nodes have the capability of extracting and metabolizing HYA (Fraser et al. 1988).

A more direct proof that intact HYA molecules leave the joint cavity via the lymph was obtained when afferent lymph vessels draining the knee joints of sheep were cannulated and HYA with approximately the same molecular weight as synovial HYA was isolated from the lymph (J R E Fraser, T C Laurent, R N P Cahill and W G Kimpton, unpublished). Furthermore, when tritium labeled HYA was injected into the joint cavity it could be identified undegraded in the lymph fluid.

Although these studies qualitatively identify lymph transport as one mechanism for removal of HYA from the joints it does not give a quantitative estimate of its importance. By the use of ^{125}I -labeled tyramine cellobiose-substituted HYA it is possible to identify the site of degradation (Dahl et al. 1988). After uptake and degradation of the HYA the labeled tyramine cellobiose is accumulated within the cells which have degraded the polysaccharide. Twenty-four hours after this compound had been injected into rabbit

knee joints 33% of the label was recovered as low-molecular weight material in the synovium and 16% in the liver (Laurent et al. 1992). A considerable amount of the injected polysaccharide must therefore have been carried via the lymph to the general circulation and taken up by the liver.

The content of hyaluronan in normal and arthritic joints

Going back to old literature (Sundblad 1965, Seppälä 1964, Balazs et al. 1967, Dahl et al. 1985) there is a large documentation on the concentration of HYA in rheumatoid synovial fluid. The concentrations recorded are usually lower in the diseased joints but due to the large increase in volume of the fluid the total amount of HYA is increased. Combining the data of Balazs et al. (1967) and Dahl et al. (1985) gives concentrations of HYA in joint fluid from normal human knees of 1.45–2.94 mg/ml (mean 2.26; n=13) and from RA knees of 0.19–1.88 mg/ml (mean 0.82; n=18). The total content of HYA withdrawn with the fluid from RA knees was 1.7–56.4 mg (mean 22.3 mg; n=17). This should be compared with an average of 2.0 mg withdrawn from normal joints and another 1.7 mg which could be recovered after washings (Balazs et al. 1967). There is also a drop in average molecular weight of HYA from $6-7 \times 10^6$ in the normal joint to $3-5 \times 10^6$ in the RA joint (Balazs et al. 1967, Dahl et al. 1985). Both the drop in concentration and the drop in molecular weight leads to a considerable drop in viscosity of the synovial fluid.

Recently we have also got some insight into the changes of HYA in the synovium in diseased state. Several groups have stained normal and rheumatoid synovium (Worrall et al. 1991, Wells et al. 1992, A Engström-Laurent, C Laurent, H Hedin and S Hellström, unpublished). In the normal tissue the main deposit seems to be in the lining layer while in the swollen inflamed synovium the polysaccharide is distributed throughout and especially in the perivascular spaces. Pitsillides et al. (1994) have also measured chemically the amount of HYA and found that normal synovium contains 1.07 ± 0.16 (SE) mg HYA/cm³ while the rheumatoid synovium contains less or 0.71 ± 0.10 mg/cm³. The concentration differences in the synovium thus mirrors those in the synovial fluid. Interestingly, a larger fraction of the HYA in the rheumatoid synovium (85%) was extracted in physiological buffer than in the normal tissue (65%). The HYA was apparently more "free" or "mobile" in the RA synovium.

Turnover rate of hyaluronan in joints

The turnover of HYA in eye of experimental animals

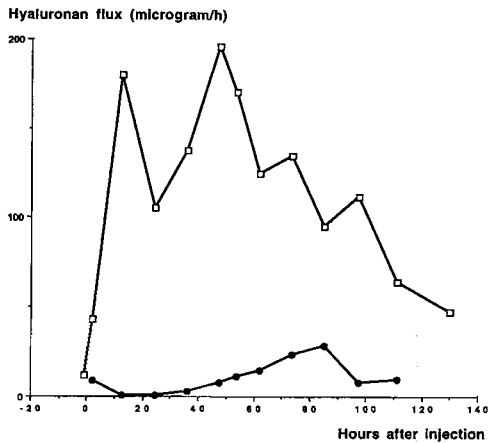


Figure 1. An immunological inflammatory reaction was induced at time 0 in the skin of a sheep. Lymph was tapped from the inflammatory region (the popliteal lymph node; \square) and from a control region (prescapular lymph node; \bullet). Volume flows and hyaluronan concentrations were recorded and the hyaluronan fluxes in the lymph were calculated. The hyaluronan flux from the inflammatory region increased close to 20-fold over the base line value. A small rise in hyaluronan flux was also recorded in the control lymph 2-3 days after start of the experiment.

has recently been determined with a technique based on injection of tritium labeled polymer locally and recording the concentration of $^3\text{H}_2\text{O}$ in plasma (Laurent et al. 1988). The rate of appearance of labeled water reflects the rate of cellular uptake and degradation of the polymer. When this technique was used on rabbit knee joints (Brown et al. 1991) using two different samples of labeled HYA with molecular weights of $> 6 \times 10^6$ and 9×10^4 half-lives of 13.2 and 10.2 h, respectively, were recorded. This is in agreement with the estimated turnover rate of tyramine-cellobiose HYA in rabbit joint (Laurent et al. 1992) and also with other estimates (Brown et al. 1991).

The technique to use ^3H -HYA to measure turnover was also applied on hock joints of sheep (Fraser et al. 1993). The sheep had been sensitized to collagen type II. In a first experiment the mean half-life of HYA was estimated in the right hock joint to be 20.8 h (15.8-27.9 h; $n=5$). Twenty-five days later collagen type II was injected in the left hock joint which induced an acute arthritis. When the mean half-life of HYA was measured in this joint 18 h later it was 11.5 (9.0-16.8) h. Thus at least an acute arthritis led to a significant increase in turnover rate.

Hyaluronan in lymph from inflammatory foci is elevated

Although the following experiment (T C Laurent and

J Hay, unpublished) was carried out on skin it demonstrates the effect of inflammatory response on HYA turnover.

Sheep were sensitized to tuberculin by injection of complete Freund's adjuvans in the skin in the popliteal region. After an appropriate time the efferent lymph vessel from the popliteal node was cannulated and lymph tapped from the region of the granuloma. At the same time efferent lymph from a prescapular lymph node was collected as a control. Lymph was collected from the two sources to obtain base line values before 60 μg of tuberculin was injected into the granuloma causing a delayed type hypersensitivity reaction. The lymph was then collected for six days and variations in HYA flux recorded. The results are described in Figure 1.

Within a few hours the popliteal lymph flow doubled and the HYA concentration increased 7-fold. This dramatically high HYA flux continued for several days and started to decline first towards the end of the week. Apparently, increased HYA turnover and presumably also increased HYA production was part of the inflammatory response. Interestingly, very little happened to the HYA-flux from the prescapular region during the first two days but then the HYA concentration slowly rose during two days before it fell again. This latter behavior could possibly be interpreted as a generalized reaction towards the local inflammation.

Discussion

Pathologically high serum levels of HYA may in principle be due either to an increased influx of HYA into the circulation or a decreased clearance. It is not possible a priori to exclude the possibility that the inflammatory condition in the joints exerts a secondary influence on the function of the liver and its ability to metabolize HYA. However, two independent studies have demonstrated that clearance of circulating HYA in patients with rheumatoid arthritis is unaffected or enhanced rather than impaired (Fraser et al. 1986, Lindqvist et al. 1992). Thus the elevated levels in serum HYA in RA patients must be due to an increased inflow.

The influx of HYA could originate in the affected joints and/or other tissues. At present the evidence favors the inflamed joints as the main source. It has been shown, at least in sheep and rabbit, that HYA can leave the joint cavity in intact form via lymph and can be detected in lymph (J R E Fraser et al., unpublished) lymph nodes (Antonias et al. 1973) and liver (Laurent et al. 1992). The experiments with tyramine

cellobiose HYA show that at least 16% of the HYA in the normal joint passes into the circulation on its way to the liver.

All experiments indicate that the amount of HYA transported via the lymph from rheumatoid joints should be elevated. Not only is the total amount of HYA highly increased in the diseased joint (Balazs et al. 1967, Dahl et al. 1985) but the half-life of the polymer may also be shorter (Fraser et al. 1993). Furthermore, the concentration of HYA in the synovium is lower and much more mobile (Pitsillides et al. 1994) which facilitates transport from the joint cavity to the lymph vessels. With a 10-fold increase in total amount of synovial HYA and a doubling of its turnover rate the lymphatic flux of HYA should increase at least one order of magnitude. It is therefore interesting to find just this kind of a reaction in an inflammatory condition in skin (Fig. 1). However, it should be noted that there is a remote possibility that the local metabolism of HYA in the rheumatoid joint is accelerated and takes care of the excess HYA.

Other indirect evidence that the excess serum HYA in rheumatoid patients originates from the musculoskeletal system is the observation that physical activity pumps HYA into the circulation and that the amount which can be recorded in serum is related to the degree of stiffness of the joints (Engström-Laurent and Hällgren 1987). However, even if it is very attractive to relate morning stiffness to an accumulation of HYA, causing an edema in the joints, it is not excluded that HYA could be deposited elsewhere, e.g. in muscles. It is to be noted that the inflammatory reaction in the popliteal region described in Fig. 1 also gave a secondary, although smaller, generalized HYA production in the same animal.

Why do we have an increased HYA turnover in the inflamed tissues? The most obvious reason is an increased production of the polysaccharide. Numerous investigations have shown that various inflammatory mediators enhance the synthesis of HYA by fibroblasts and other cells. Among these mediators are cAMP, prostaglandins, interleukin 1, interferon, TNF- α , lipopolysaccharides and various growth factors such as PDGF, EGF, TGF β and bFGF, which may operate in the inflammatory area (for early references see Laurent and Fraser 1986, Wells et al. 1992, Heldin et al. 1989). The biological significance of this activation of HYA biosynthesis is not known. There is also another mechanism, which may increase the lymph flux of HYA. Several investigations have shown that an increased fluid flux through the tissues leads to an increased washout of hyaluronan into the circulation (for ref. see Reed and Laurent 1992).

Acknowledgements

Supported by grants from the Swedish Medical Research Council (3x-4), Gustaf V 80-year Birthday Fund, the Australian National Health and Medical Research Council and the Buckland Foundation.

References

- Andersson Gäre B, Fasth A. Serum concentration of hyaluronan, IgM and IgA rheumatoid factors in a population based study of juvenile chronic arthritis. *Scand J Rheumatol* 1994; 23: 183–90.
- Antonias K N, Fraser J R E, Muirden K D. Distribution of biologically labelled radioactive hyaluronic acid injected into joints. *Ann Rheum Dis* 1973; 32: 103–11.
- Balazs E A, Watson D, Duff I F, Roseman S. Hyaluronic acid in synovial fluid. Molecular parameters of hyaluronic acid in normal and arthritic fluids. *Arthr Rheum* 1967; 10: 357–76.
- Björk J, Kleinau S, Tengblad A, Smedegård G. Elevated levels of serum hyaluronate and correlation with disease activity in experimental models of arthritis. *Arthr Rheum* 1989; 32: 306–11.
- Brown T J, Laurent U B G, Fraser J R E. Turnover of hyaluronan in synovial joints: elimination of labelled hyaluronan from the knee joint of the rabbit. *Exp Physiol* 1991; 76: 125–34.
- Chassagne P, Le Loet X, Delpech B, Levesque H, Menard J F, Maingonnat C, Deshayes P. Étude des variations nyctérmérales du taux sérique d'acide hyaluronique au cours de la polyarthrite rhumatoïde. *Rev Rhum* 1989; 56: 775–9.
- Dahl L B, Dahl I M S, Engström-Laurent A, Granath K. Concentration and molecular weight of sodium hyaluronate in synovial fluid from patients with rheumatoid arthritis and other arthropathies. *Ann Rheum Dis* 1985; 44: 817–22.
- Dahl L B, Laurent T C, Smedsrod B. Preparation of biologically intact radioiodinated hyaluronan of high specific radioactivity: coupling of 125 I-tyramine-cellobiose to amino groups after partial N-deacetylation. *Anal Biochem* 1988; 175: 397–407.
- Engström-Laurent A, Hällgren R. Circulating hyaluronate in rheumatoid arthritis: relationship to inflammatory activity and the effect of corticosteroid therapy. *Ann Rheum Dis* 1985; 44: 83–8.
- Engström-Laurent A, Hällgren R. Circulating hyaluronic acid levels vary with physical activity in healthy subjects and in rheumatoid arthritis patients. *Arthr Rheum* 1987; 30: 1333–8.
- Engström-Laurent A, Laurent T C. Hyaluronan as a clinical marker. In: *Clinical impact of bone and connective tissue markers*. (Eds. Lindh E, Thorell J I) Academic Press, London 1989: 235–52.
- Engström-Laurent A, Laurent U B G, Lilja K, Laurent T C. Concentration of sodium hyaluronate in serum. *Scand J Clin Lab Invest* 1985; 45: 497–504.
- Fraser J R E, Engström-Laurent A, Nyberg A, Laurent T C. Removal of hyaluronic acid from the circulation in rheumatoid disease and primary biliary cirrhosis. *J Lab Clin Med* 1986; 107: 79–85.

- Fraser J R E, Kimpton W G, Laurent T C, Cahill R N P, Vakakis N. Uptake and degradation of hyaluronan in lymphatic tissue. *Biochem J* 1988; 256: 153-8.
- Fraser J R E, Kimpton W G, Pierscionek B K, Cahill R N P. The kinetics of hyaluronan in normal and acutely inflamed synovial joints: observations with experimental arthritis in sheep. *Sem Arthr Rheum (Suppl. 1)* 1993; 22: 9-17.
- Fraser J R E, Laurent T C, Pertoft H, Baxter E. Plasma clearance, tissue distribution and metabolism of hyaluronic acid injected intravenously in the rabbit. *Biochem J* 1981; 200: 415-24.
- Goldberg R L, Huff J P, Lenz M E, Glickman P, Katz R, Thonar E J-M A. Elevated plasma levels of hyaluronate in patients with osteoarthritis and rheumatoid arthritis. *Arthr Rheum* 1991; 34: 799-807.
- Heldin P, Laurent T C, Heldin C-H. Effect of growth factors on hyaluronan synthesis in cultured human fibroblasts. *Biochem J* 1989; 258: 919-22.
- Laurent T C, Fraser J R E. The properties and turnover of hyaluronan. In: *Functions of the proteoglycans*. Ciba Found Symp 124. Wiley, Chichester 1986: 9-29.
- Laurent T C, Fraser J R E. Hyaluronan. *FASEB J* 1992; 6: 2397-2404.
- Laurent T C, Lilja K, Brunnberg L, Engström-Laurent A, Laurent U B G, Lindqvist U, Murata K, Ytterberg D. Urinary excretion of hyaluronan in man. *Scand J Clin Lab Invest* 1987; 47: 793-9.
- Laurent U B G, Fraser J R E, Engström-Laurent A, Reed R K, Dahl L B, Laurent T C. Catabolism of hyaluronan in the knee joint of the rabbit. *Matrix* 1992; 12: 130-6.
- Laurent U B G, Fraser J R E, Laurent T C. An experimental technique to study the turnover of concentrated hyaluronan in the anterior chamber of the rabbit. *Exp Eye Res* 1988; 46: 49-58.
- Laurent U B G, Laurent T C. On the origin of hyaluronate in blood. *Biochem Internat* 1981; 2: 195-9.
- Levesque H, Delpech B, Le Loet X, Deshayes P. Serum hyaluronate in rheumatoid arthritis: study by affinity-immunochemical assay. *Br J Rheumatol* 1988; 27: 445-9.
- Lindqvist U, Engström-Laurent A, Laurent U, Nyberg A, Björklund U, Eriksson H, Pettersson R, Tengblad A. The diurnal variation of serum hyaluronan in health and disease. *Scand J Clin Lab Invest* 1988; 48: 765-70.
- Lindqvist U, Groth T, Lööf L, Hellsing K. A hyaluronan-loading test applied to patients with liver and joint diseases. *Clin Chim Acta* 1992; 210: 119-32.
- Lindqvist U, Laurent T C. Serum hyaluronan and aminoterminal propeptide of type III procollagen: variation with age. *Scand J Clin Lab Invest* 1992; 52: 613-21.
- Paimela L, Heiskanen A, Kurki P, Helve T, Leirisalo-Repo M. Serum hyaluronate level as a predictor of radiologic progression in early rheumatoid arthritis. *Arthr Rheum* 1991; 34: 815-21.
- Pitsillides A A, Worrall J G, Wilkinson L S, Bayliss M T, Edwards J C W. Hyaluronan concentration in non-inflamed and rheumatoid synovium. *Br J Rheum* 1994; 33: 5-10.
- Poole A R, Witter J, Roberts N, Piccolo F, Brandt R, Paquin J, Baron M. Inflammation and cartilage metabolism in rheumatoid arthritis. *Arthr Rheum* 1990; 33: 790-9.
- Reed R K, Laurent U B G. Turnover of hyaluronan in the microcirculation. *Am Rev Respir Dis* 1992; 146: S37-S39.
- Saari H, Kontinen Y T, Nordström D. Effect of joint mobilization on serum hyaluronate. *Ann Med* 1991; 23: 29-32.
- Seibel M J, Raimann S, Seglias J, Lautenschläger J, Müller W. Serum-Hyaluronsäure und aminoterminals Prokollagen-III-Peptid bei entzündlichen und degenerativen Gelenkerkrankungen. *Z Rheumatol* 1988; 47: 98-106.
- Smedsröd B, Pertoft H, Eriksson S, Fraser J R E, Laurent T C. Studies in vitro on the uptake and degradation of sodium hyaluronate in rat liver endothelial cells. *Biochem J* 1984; 223: 617-26.
- Seppälä P. Synovial fluid in rheumatoid arthritis. Turku: University of Turku, 1964 (Thesis).
- Sundblad L. Glycosaminoglycans and glycoproteins in synovial fluid. In: *The amino sugars*. (Eds. Balazs E A, Jeanloz R W) Academic Press, New York and London 1965: 2A: 229-50.
- Wells A F, Klareskog L, Lindblad S, Laurent T C. Correlation between increased hyaluronan localized in arthritic synovium and the presence of proliferating cells. *Arthr Rheum* 1992; 35: 391-6.
- Worrall J G, Bayliss M T, Edwards J C W. Morphological localization of hyaluronan in normal and diseased synovium. *J Rheumatol* 1991; 18: 1466-72.