The complexity of the mechanisms of action of hyaluronan in joint diseases

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Hyaluronan or hyaluronic acid (HA) molecules are long, linear polysaccharide chains composed of a disaccharide repeating unit of N-acetyl glucosamine and glucuronic acid. HA is a major component of the articular tissues, where it is thought to be essential in the protection of articular cartilage and the transport of nutrients to cartilage. Changes in the properties of HA are associated with most joint diseases and may contribute to the extent and severity of the pathological process (1).

Based on the hypothesis that the intra-articular (IA) injection of a ‘good’ HA could restore at least in part the properties of the synovial fluid (SF), this treatment was first tested more than 20 years ago in human osteoarthritis (OA) by Peyron and Balazs (2). Since then many controlled studies have been performed, most of these with Hyalgan®, reporting clinical improvement and the superiority of HA in comparison with placebo and other IA therapies in OA of the knee (3-8). The paper by E. Tamir et al. published in this issue of Clinical and Experimental Rheumatology (9) seems to confirm previous observations of the usefulness of treatment with IA HA.

However, despite this strong body of evidence and the increasing number of people now receiving such therapy all over the world, doubts still exist with regard to the mechanisms of action of HA. Some of these remain ‘wrapped in mystery’, and the decision to use HA thus seems sometimes to be derived from empirical rather than scientific considerations. The frequent discrepancies between the effects of HA in vitro or in animals and those observed in humans could explain, at least in part, this attitude.

In an attempt to define a profile of the mechanisms of HA actions in arthropathies, three main groups of effects could be proposed: (1) effects on joint mechanics; (2) effects on inflammation; (3) effects on the pathophysiology of OA or structural effects on OA.

Effects on joint mechanics

In joints, HA is synthesized by the enzyme HA synthase (HAS), located in the plasma membrane of chondrocytes and type B synoviocytes (10). The gene sequence for the enzyme responsible for the synthesis of HA has been identified and cloned. Thus, 3 different HAS have been described in eukaryotic cells – termed HAS-1, HAS-2 and HAS-3 (11) – and recently it has been demonstrated that HAS-2 is the enzyme predominantly involved in the synthesis of HA in human and bovine articular cartilage (12). HAS may be regulated by many agents, including growth factors, interleukin-1 (IL-1) (13) and HA itself (14, 15). In joints, while the HA synthesized by type B synoviocytes is mainly released in the SF, that produced by the chondrocytes becomes integrated in the cartilage matrix where it is essential in maintaining the structure of the aggrecan. HA influences the hydration of the aggrecan molecules necessary to confer the functions of resilience and elastic strength to cartilage, thus facilitating its load-bearing functions. Structural changes in HA, in particular involving its chain length and molecular weight (MW), also impair some important functions of SF, such as shock absorption, traumatic energy dissipation, and the storage and lubrication of cartilage.

It is not surprising that the main functions attributed to HA were first thought to be linked to its unique rheological properties, although recent studies on the lubricin – the boundary layer lubricant of synovial joints (16) – have raised some doubts regarding the actual role of the HA in joint lubrication (17). Since a reduction in the concentration and MW of HA influences the viscosity of SF and its capacity to store mechanical energy and permit the increase of frictional resistance, one of the first indications for the use of HA was to compensate for the low MW and concentrations found in arthropathies in order to improve the mechanics of joint movements (18).

This is in keeping with the concept of ‘viscosupplementation’ first proposed by Balazs in 1960 (19) and based on the hypothesis that the intra-articular injection of HA in OA could restore the physiological homeostasis of the joint by improving the rheological properties of the SF and promoting the syn-
thesis of an endogeneous HA with higher MW and possibly more functionality. In addition, it has been suggested that the equilibrium between the rigid and flexible segments of HA chains is crucial to maintain the viscoelastic properties of HA (20). This balance may be disturbed with an increase in the flexibility of the HA chains, determined by their phospholipid content, which is in turn influenced by the excessive amounts of phospholipids found in arthopathies (21).

**Effects on inflammation**

The anti-inflammatory effects of HA have been suggested by number of studies (Table I). Unfortunately, as most of them were performed in vitro or in animals, they are frequently insufficient to adequately reproduce the complexity of the inflammatory process in man. It is possible that HA acts on particular aspects of the inflammatory process and that its effects can be influenced by the type of arthropathy and, on the other hand, by the MW or the concentration of HA itself.

An important ‘basic’ effect may be exerted on synovial membrane, where the HA may control the cellular traffic between the vascular and interstitial compartments, which include SF. It has been observed that HA inhibits the migration, chemotaxis, phagocytosis and adherence of leukocytes (20, 22) and may also influence the production of some of the important inflammatory mediators found in SF (23). Furthermore, other important effects were observed on the reactive oxygen derived radical species (RORS) which are among the elements most involved in HA depolymerisation in SF (24, 25). HA itself or its derivative subcomponent D-glucuronic acid may in turn have a scavenging function in the joint, playing an active role in the protection of the articular tissues from oxidative damage (26). In addition, HA has been demonstrated to be effective in reducing cell cartilage and chondrocyte injury due to degradative enzymes, RORS and interleukin (IL)-1 in explanted bovine cartilage and isolated chondrocytes (27). Interestingly, when superoxide dismutase (SOD) from bovine erythrocytes was conjugated with HA with a mean MW of 10⁶, the resulting complex exhibited much higher anti-inflammatory activities than HA or SOD in models of inflammatory diseases such as ischemic oedema of the foot-pad in mice, and carrageenin-induced pleurisy and adjuvant arthritis in rats (28).

Effects on pro-inflammatory cytokines and metalloproteinases (MMPs) also play a crucial role in inflammation. An influence of HA on IL synthesis and/or production has been found in some studies performed in animals (29) and in humans (23, 30, 31). In one study on

<table>
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<tr>
<th>Table I. Main effects of HA on inflammation.</th>
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<tr>
<td>Type of effect</td>
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<tr>
<td>Inhibition of leukocyte migration</td>
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<tr>
<td>Inhibition of leukocyte phagocytosis</td>
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<tr>
<td>Inhibition of neutrophil adhesion</td>
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<tr>
<td>Inhibition of lymphocyte proliferation</td>
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<tr>
<td>Inhibition of synovial cell proliferation</td>
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<tr>
<td>Prevention of reactive-oxygen derived radical species (RORS) damage</td>
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<tr>
<td>Inhibition of PGE2 or PGF2 production</td>
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<tr>
<td>Stimulation of cAMP production</td>
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<tr>
<td>Inhibition of carrageenin oedema</td>
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<tr>
<td>Inhibition of adjuvant arthritis</td>
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<tr>
<td>Inhibition of metalloproteinase expression or production</td>
</tr>
<tr>
<td>Inhibition of IL-1 synthesis or production</td>
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<tr>
<td>Inhibition of IL-6 production</td>
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<tr>
<td>Inhibition of IL-8 production</td>
</tr>
<tr>
<td>Inhibition of TNF production</td>
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<tr>
<td>Modification of CD44 expression</td>
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**Fig. 1.** Effects of 4 intra-articular injections of sodium hyaluronate (Hyalgan, once weekly) on synovial fluid levels of IL-1 in patients with rheumatoid arthritis. *p < 0.05 vs basal (week 0) values.
RA patients with synovial effusions, we administered a cycle of 4 HA injections; while the first two injections tended to slightly increase SF levels of IL-1, at the final observations the levels of this proinflammatory cytokine were significantly lower than the basal values (23) (Fig. 1). This study led us to hypothesise that HA may act as a cyto-modulating drug and it is possible that this modulation is mediated by the HA specific membrane receptor which belongs to the CD44 family (32). Since many cells carry this receptor, HA may in this way influence various cell interactions. Concerning the cytokines, it has been demonstrated that IL-1 may up-regulate CD44 expression (mRNA and protein) in cartilage explants (13). In turn, in a model of rat air pouch induced by sensitization with lipopolysaccharide, HA increased the percentage of CD44+ cells and reduced the concentrations of IL-1 and tumor necrosis factor (TNF) in the exudate, thus suggesting that HA might affect the inflammatory process by modifying CD44 expression on infiltrating cells in air pouch exudate (33). In addition, the inhibition of TNF production by inflammatory cells and the modulating effects of HA in two models of acute and chronic inflammation in rats may be explained by the interaction of CD44 and HA (34).

Concerning its possible influence on MMPs, HA was found to suppress the expression of MMP-3 in animals (29, 35). Furthermore, in two preliminary studies performed in humans, we observed that intra-articular HA (Hyalgan) was able to reduce the SF levels of MMP-1 and MMP-3, and in addition there was a trend towards an increase in the SF TIMP-1 levels one week after the injections (36, 37).

Other important effects are those on prostanoids. We first reported that a single injection of Hyalgan may reduce the levels of prostaglandin (PG)E2 and increase the levels of cAMP in SF of various arthropathies (38). Subsequently, this type of effect on PGE2 was confirmed by two other studies performed in vivo (39, 40). Furthermore, it has recently been reported that HA injections reduced the total amounts of IL-1, leukotriene C4, 6-keto-PG1 and PGF2 in the SF of temporomandibular joints (41). Interestingly, HA was able to inhibit in a dose-related fashion two standard models of acute and chronic inflammation in the rat, i.e. carrageenin oedema and adjuvant arthritis (42).

However, despite these important actions on several inflammatory mediators, the small amount of evidence showing any efficacy of IA HA in human inflammatory arthropathies (43) have not encouraged its use. This might be explained by the slow clinical effects of HA injections in these conditions, which are considered unsatisfactory particularly when compared to those usually observed with steroids.

Pathophysiological and structural effects on OA
It has been suggested that HA may influence the evolution of OA by increasing HA synthesis from synovial cells (44) and reducing keratan sulfate levels in the SF of OA (45). It has been shown that exogenous HA was able to restore the proteoglycan content of OA human cartilage previously damaged by fibronectin (46). By this anabolic activity, in addition to the above mentioned anticitabolic effects, particularly against MMPs, exogenous HA may influence the progression of OA, as has been observed in experimentally-induced OA (47-51). However, it is possible that a longer persistence of HA and/or a high number of injections are necessary to produce effects on cartilage in humans, in accordance with its “slow acting” or “chondroprotective” role. Some preliminary long-term studies are, in this context, encouraging (3, 50, 52, 53).

In keeping with a possible structural effect of HA on OA is a recent study reported by Frizziero et al. (54), using microarthroscopy and biopsy procedures. These authors demonstrated a significant improvement in synovial and cartilage changes of knee OA 6 months after a cycle of 5 weekly injections of Hyalgan. In particular, compared to baseline a reconstitution of the superficial amorphous layer of the cartilage, an improvement in the chondrocyte density and vitality, and a reduction in synovial inflammation accompanied by a significant increase in the synovial repair process were observed. Similar findings were reported by Listrat et al. (54), who used arthroscopy in a randomized, controlled, pilot study comparing HA to conventional therapy, to evaluate the effects of Hyalgan on the chondral lesions of 39 patients with OA of the knee (55). A cycle of 3 injections over a period of 2 weeks was repeated every 3 months for a total of 3 cycles. At 1 year from baseline, patients treated with HA demonstrated greater improvement in their quality of life and less progression of chondropathy in comparison with the control group.

Other effects
However, since the lag-time before any structural effects are seen is relatively long, the rapid symptomatic effects frequently found in patients with OA of the knee should be probably attributed to other, probably anti-inflammatory or anti-hyperalgesic, mechanisms. In addition to their influence on PGs and cAMP (38), another direct analgesic effect of HA has been reported in a rat model of OA induced by the injection of bradykinin (56). Furthermore, it is worth noting that HA may influence by means of viscosupplementation the response levels of the sensory afferent fibers and nociceptors located in synovial and subsynovial tissue (19, 57).

In keeping with these observations, in recent clinical studies HA injections were found to be as effective as non-steroidal anti-inflammatory drugs (NSAIDs) on the parameters of pain and function in knee OA, but with fewer gastrointestinal side effects (7, 58).

The molecular weight dilemma
The MW of HA preparations currently available for IA therapy may vary considerably, ranging from 0.5 to 6 million (Table II). These latter high MW products are cross-linked forms of HA, generically termed hylans. In accordance with the concept of viscosupplementation may be the assumption that the greater the MW of the injected HA, the greater its therapeutic usefulness.
However, at present there is no demonstration that the effects of intra-articular HA depend exclusively on mechanisms related to its physical properties. Furthermore, a correlation between MW and efficacy is not borne out by an analysis of the available literature on clinical results, SF viscosity, HA concentration, HA MW and the rate of synthesis in joint disease (59). Thus, it is possible that the beneficial effects of HA in joint diseases may be due to pharmacological rather than physical properties and that additional or independent mechanisms are responsible, including anti-inflammatory and structure modifying effects. In keeping with such a hypothesis is a recent report comparing the access of two different HAs to synoviocytes; HA with a MW of 500-730 kDa was found to be broadly distributed in the synovial lining layer while HA with a MW of 2300 kDa was almost undetectable in the tissue (60).

Conclusions

Although a number of studies have elucidated many functions related to the effects of HA, the mystery of the precise mechanisms of the therapeutic action of exogenous HA remains at least in part still unresolved. However, it is undeniable that in recent years the use of intra-articular injections of HA in the treatment of OA has significantly increased world-wide. It is probable that most decisions to use this drug to treat patients with OA are derived from its therapeutic benefits associated with a remarkable safety profile.

References


Table II. Current hyaluronan preparations available for intra-articular therapy of osteoarthritis.

<table>
<thead>
<tr>
<th>HA preparation</th>
<th>Manufacturer</th>
<th>Weight-average molecular weight</th>
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<tbody>
<tr>
<td>Hylan®*</td>
<td>Fidia (Italy)</td>
<td>500-730 kDa</td>
</tr>
<tr>
<td>Supluxyn®</td>
<td>Bioniche (Canada)</td>
<td>500-730 kDa</td>
</tr>
<tr>
<td>(Sup)Artz®</td>
<td>Seikagaku (Japan)</td>
<td>600-1200 kDa</td>
</tr>
<tr>
<td>Adant®*</td>
<td>Meiji (Japan)</td>
<td>600-1200 kDa</td>
</tr>
<tr>
<td>Orthovisc®</td>
<td>Anika Therapeutics (USA)</td>
<td>1500 kDa</td>
</tr>
<tr>
<td>Ostenil®</td>
<td>Chemedica (Germany)</td>
<td>1300-1600 kDa</td>
</tr>
<tr>
<td>Fermathron®</td>
<td>Fermenotech (UK)</td>
<td>1500-2000 kDa</td>
</tr>
<tr>
<td>Go-on®</td>
<td>Rottapharm (Ireland)</td>
<td>1800-2400 kDa</td>
</tr>
<tr>
<td>Arthrum®</td>
<td>LCA Pharmaceutical (France)</td>
<td>2400 kDa</td>
</tr>
<tr>
<td>Synvisc®*</td>
<td>Biomatrix (USA)</td>
<td>&gt; 6000 kDa (chemically cross-linked HA)</td>
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*Available in the U.S.
**EDITORIAL**

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