European Review for Medical and Pharmacological Sciences 2014; 18: 3326-3338

Viscosupplementation with high molecular weight native hyaluronan. Focus on a 1500-2000 KDa fraction (Hyalubrix®)

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Abstract. – BACKGROUND: Joint disease reduces the rheological properties of synovial fluid, increasing the susceptibility of the articular cartilage to damage. Thus, a therapeutic strategy, called viscosupplementation, was proposed in which intra-articular injections of a suitable material are used to restore the viscoelastic properties of the synovial fluid. Solutions of high-MW native HA, which is the main component of the synovial fluid, were a natural choice to reach this goal, but HA-derived materials, engineered to achieve greater elastoviscosity and intra-articular residence time, were also developed. In the last twenty years the clinical experience showed that viscosupplementation is effective in joint diseases such as osteoarthritis, with beneficial effects on pain, function and patient global assessment. However, a marked variability between different preparations on different outcome parameters was reported to exist.

AIM: In the present paper the available data on Hyalubrix®, a specific 1.5% formulation of natural HA with MW in the range 1500-2000 kDa, were reviewed, trying to outline, in the framework of the available intra-articular therapies, the role it can play for the symptomatic management of patients with degenerative joint arthropathy.

Keywords:
Hyaluronan, Osteoarthritis, Intra-articular therapy, Viscosupplementation, Viscoinduction.

Introduction

Hyaluronan (also referred to as hyaluronic acid (HA) or sodium hyaluronate) is the most abundant glycosaminoglycan in mammalian tissue. It is present in high concentrations in connective tissue, such as skin, vitreous humor, cartilage, and umbilical cord, but the largest single reservoir is the synovial fluid (SF) of the diarthrodial joints, where concentrations of 0.5-4 mg/mL are achieved[1,2]. The high concentration of HA in SF is essential for normal joint function, because HA confers exceptional visco-elasticity and lubricating properties to SF, particularly during high shear conditions. Under dynamic loading of diarthrodial joints, shear thinning and a reduction in viscosity occur because of decreased physical entanglements of HA molecules and their realignment to directions more parallel with the axis of articulation. These unique non-Newtonian rheological properties of HA not only reduce wear and attrition of articular cartilage during joint motion[3-5] but also stabilize joints at low shear rates[6].

In addition, the structural characteristics of HA molecules restrict the entry of large plasma proteins and cells into the aqueous phase of SF[7,8]. By contrast, HA facilitates the transport of water and small solutes through SF to articular cartilage from capillaries in the synovium and reduces fluid loss as intra-articular pressure is raised during joint flexion[9,10].

The diverse physicochemical properties of HA arise from its unique macromolecular structure, an exceptionally long chain (up to 30 μm) of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. Despite the simplicity of its primary structure, this linear polysaccharide can adopt highly coiled conformations in solution. In the joint cavity the HA molecules are mainly synthesized by the type B synoviocytes, that release a polydispersed HA population with molecular weight (MW) ranging between $2 \cdot 10^6$ and $10 \cdot 10^6$ Da[1,2,11]. Moreover, HA is produced in large quantities, leading to the formation of extensive macromolecular entanglements and networks that confer to the synovial fluid its characteristic rheological properties, i.e. the elasticity and viscosity responsible for shock absorption under conditions of high compression or shear, and lubrication in low load states.

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It is well known that joint arthropathies of traumatic and degenerative nature (such as osteoarthritis) are associated with a reduction of the molecular weight and concentration of hyaluronan in the synovial fluid. In fact, the presence of proinflammatory cytokines, free radicals and proteinases in the synovia can adversely affect the metabolism of the lining type B fibroblasts, leading to the biosynthesis of HA with abnormal MW, as has been shown by analysis of synovial fluid from pathologic joints[5,11,12]. In addition, HA also may be depolymerized by oxygen-derived free radicals[13] and intracellularly by hyaluronidases, and other glycosidases from synoviocytes and leukocytes in the synovium[14,15]. The decline in HA molecular size coupled with its dilution by infiltration of plasma fluid and proteins (caused by increased synovial membrane permeability) reduce the rheological properties of synovial fluid from diseased joints[5,11-13]. As a consequence, it was contended that cartilage attrition and subchondral bone remodeling was enhanced contributing to progression of pathology and clinical symptoms.

Based on these findings, more than thirty years ago Balazs and Denlinger[16] introduced the concept of *viscosupplementation*, a therapeutical approach to osteoarthritis (OA) involving the replacement of the SF with highly purified HA to restore (or supplement) SF viscoelasticity, to decrease symptoms, and improve joint functionality. The clinical studies undertaken to test this hypothesis were in general supportive in terms of relief of OA symptoms[16-21], but also raised new questions. In particular, it was observed that the clinical outcomes obtained by this approach also could be achieved with HAs with MWs less than the HA in SF aspirated from the defective joint[22-25]. Moreover, the clinical effectiveness of the therapy was often lasting several weeks or even months, which was inconsistent with the known rate of clearance of HA of this size from the synovial joint[26-28]. To overcome this inconsistency Ghosh and Guidolin[29] suggested that the mechanism of action of intra-articular HA was dependent on MW. The biological basis of this proposal relies on the type of interaction HAs of different MW establish with the specific HA-receptors (in particular CD44) at the cell membrane. In general, the consequence of these receptor interactions by HA is to stimulate transduction and other signaling pathways that modulate cell functional activities manifested primarily by cell migration, proliferation, and endocytosis[30]. Studies (see[29,31] for a review) on the effect of HAs on CD44 cellular signaling have shown that the results depend on the size of the HA molecules used, low MW-HAs being in general more efficient. For instance, low MW-HA (0.20 x 10^6 Da) was shown to be more effective in maintaining the survival of blood eosinophils than HA with an MW of $3x10^6$ Da[32]. The authors proposed that this protective effect was mediated via the increased expression of granulocyte macrophage colony-stimulating factor after CD44 activation. The mechanisms responsible for this MW-dependent action of HA on its receptors are still not fully understood, but, at least in part, may be related to the clustering and cross-linking of CD44 on the plasma membrane[29,33]. In fact, it has been recently shown that HA preparations within a specific size range can provoke a pattern of CD44 clustering and crosslinking on binding, which then triggers an intracellular signal, whereas the larger HA molecules may occupy these multiple CD44 linking sites but prevent receptor crosslinking and a cellular reaction[33]. Because the HA present in normal connective tissues is generally of high MW, it would seem to make biologic sense for it not to continuously stimulate an active response from the cells it surrounds. Thus, a mechanism of action which is mainly of pharmacological type (i.e. receptor-mediated) can be surmised for HA of lower molecular weight. As far as the joint tissues are concerned, this concept found experimental support in the studies by Smith and Ghosh[34], showing that human synovial fibroblasts derived from OA joints when cultured with HAs responded by upregulating or down-regulating endogenous HA synthesis, depending on the media concentration and the MW of the exogenous HA added[34]. The maximal stimulation of endogenous HA synthesis was produced by HAs with MWs around $0.5x10^6$ Da, and the cell response significantly decreased when HAs of MW of about $3x10^6$ Da were used. The term *viscoinduction*[29] was then coined to describe the main mechanism of action exploited by HA of MW between $0.5x10^6$ – $1x10^6$ Da to induce clinical benefits following intra-articular administration, whereas the *viscosupplementation* concept appears more appropriate to describe the physical mechanism of action mainly exploited by high-MW HAs and by products based on modifications of HA molecules to achieve greater elastoviscosity and intra-articular dwell-time[35]. Thus, significant differences could exist among the various HA-based formulations proposed for the intra-articular therapy of joint diseases.

The quite large amount of available data concerning the use of a specific high-MW HA formulation (Hyalubrix®) as a viscosupplement for the management of joint arthropaties will be the focus of the present review article. This agent is also referred to as Hyalubrix60® or Hyalone®.

Hyalubrix® as a viscosupplement

Hyalubrix® is a 1.5% solution of non-modified HA (15 mg/ml) obtained by biofermentation with molecular weight in the range 1500-2000 kDa[36,59]. Ideally, to fulfill the objectives of viscosupplementation[16] following intra-articular administration, an exogenous substance should exhibit a behavior very similar to the one of the synovial fluid it replaces. In particular, it has to meet some objectives:

- Proper rheological properties
- Trans-synovial fluid buffering, and permeability to metabolites and macromolecules
- Good residence time

These aspects will be here briefly discussed, and in this context available physicochemical data focused on solutions of HA with molecular weight in the range 1500-2000 kDa will be also summarized. Although not directly obtained with Hyalubrix®, these results directly concern the HA fraction used in that preparation. Thus, they can be useful in order to assess to what extent Hyalubrix® can operate as a viscosupplement.

Rheological properties

Synovial fluid acts predominantly as a viscous fluid when it is exposed to low deformation frequencies (slow movement) and behaves as an elastic shock absorber when it is subjected to a high rate of deformation, such as during running or jumping[19]. This rheological profile, which is strongly dependent on the HA content, is critical to the physiologic function of the synovial fluid. It can be characterized by evaluating how the frequency of the applied stress affects the relative values of the elastic modulus (G') and the viscous modulus (G")[37]. The strain frequency at which these two moduli intersect is called the "cross-over point" and represents the frequency at which the synovial fluid changes from predominantly viscous to predominantly elastic. When compared to healthy SF the cross-over frequency exhibited by HA preparations in the considered range of MW and concentration (~0.8 Hz) was found about twice the one observed in the joint fluid samples (~0.4 Hz), but significantly closer to it when compared to the values observed in HA preparations of lower MW (~11 Hz) also used for intra-articular therapy[38].

It has to be pointed out that endogenous HA is also involved in the lubrication of the synovial joint by two main mechanisms. From one side it is characterized by intrinsic lubricating properties[39], on the other side it can interact with phospholipids, giving rise to complexes exhibiting peculiar lubricating[40] and protective[41] characteristics. Both these features are MW-dependent and become more efficient with increasing chain length[40,41].

Trans-synovial flow

The trans-synovial flow is a very well regulated process in the joint, since even a small, sustained imbalance of it would quickly lead to joint swelling or fluid depletion. Sustained flexion is a particular threat to volume homeostasis, because it raises intra-articular pressure, driving fluid out of the joint cavity. An important function of hyaluronan in the synovial fluid is to counter this threat by "buffering" the fluid drainage rate.

It has been shown[42] that the outflow buffering effect is dependent on the HA molecular weight. In this study HAs of different MW were infused into the knees of anesthetized rabbits, with Ringer solution as control in the contralateral joint, and trans-synovial drainage rate was recorded at known joint pressures. With hyaluronan of low MW (90 kDa, 300 kDa) the fluid drainage rate was reduced relative to Ringer solution, but increased steeply with pressure, indicating that there was no outflow buffering. When HAs approaching 2000 kDa were used, the fluid drainage rate became relatively insensitive to pressure, causing a near plateau of flow. Furthermore, hyaluronan concentration in the joint cavity increased over the drainage period, indicating partial reflection of hyaluronan by synovial interstitium. Reflected fraction was of about 0.80 for HA in the MW range here considered. The last result also indicated that HA with this MW exhibits a very low diffusion in the articular tissues, and, as a consequence, a low interaction with cells and receptors. This finding is consistent with previous studies performed *in vivo* with fluoresceinated HA (see[43]) showing a minimal penetration of high-MW HA in the synovial lining following intra-articular injection.

For what it concerns the solute exchange between the synovial capillaries and cartilage and

other joint tissues it is also evident that an intra-articularly administered solution of high-MW natural HA exhibits a behavior very similar to that of SF, which is itself a HA solution[8]. This aspect is important to ensure the nutrition of articular cartilage as well as for the elimination of metabolites and noxious substances from the joint cavity.

Residence time

Data on the kinetics of HA with MW in the considered range were provided by Komatsu et al[44]. In this study the pharmacokinetics of ^{14}C-glucose-labelled HA was measured after intra-articular injection into the knee joint cavity of rabbits. After a single administration the measured half-life was of 24 hours. However, 10% of the high MW material was still present in the joint cavity after 72 hours and the radioactivity disappeared in about 120 hours. The observed half-life and residence time following a single intra-articular injection were significantly higher than the ones exhibited by HA with MW < 1000 kDa (which are of about 16 hours and 60 hours respectively[45]). A potentially interesting finding of this study concerned the pattern of degradation products generated starting from the injected high-MW HA. In fact, fragments of 300 kDa and, at a later stage, of 50 kDa were mainly found in the articular environment. They were then broken down by cells into C_1 units (carbon cycle) before being re-used as an *in vivo* constituent of the body.

Altogether the abovementioned data outline a physicochemical profile for the HA fraction used in Hyalubrix® that suggests for it mechanical properties quite close to the ones characterizing the synovial fluid, and a quite limited interaction with the cells in the joint tissues. Thus, it appears a suitable material to pursue a therapeutical strategy based on the viscosupplementation concept. In this respect, available clinical data on the use of Hyalubrix® will be reviewed in the following section.

Therapeutic efficacy

The effect of intra-articular administration of Hyalubrix® (the high-MW hyaluronic acid formulation which is the focus of the present review) has been tested in a number of controlled and observational clinical trials, that will be here briefly described.

Noncomparative trials

A multicenter, large-scale, observational study by Schieb[46] involved a total of 1523 patients, followed by 515 clinical centers. 81.3% of recruited subjects suffered from gonarthrosis, the others were affected by arthropaties of traumatic origin. They received a single cycle of three weekly intra-articular injections and were observed for a maximum of six weeks. Intensity of pain and mobility were evaluated according to a Visual Analogue Scale (VAS), both at the start and end of treatment. A significant reduction in the pain suffered by the patients was observed. Upon conclusion of administration of Hyalubrix®, 91.2% of patients reported generally much less pain than prior to beginning of treatment. An equally significant improvement was observed with regard to mobility. Towards the end of treatment, 85.3% of patients reported that limitations of mobility had either disappeared completely or, at worst, were very slight. This observational study demonstrated that a cycle of three injections was sufficient to induce a significant reduction in pain and an overall improvement in mobility. Moreover, it indicated that therapeutic benefits can be obtained not only for degenerative arthropathies, but also for arthropathies of traumatic origin in younger patients.

These findings were confirmed and extended by another large-scale, prospective and observational study, aimed at investigating the safety and efficacy of Hyalubrix® in the treatment of synovial joint OA, recently proposed by Foti et al[47]. This study was carried out at 47 specialist centers for physiatrist, orthopedics and rheumatology in Italy, and involved 1266 patients with radiologically diagnosed OA (located in the knee, hip, shoulder, tibio-tarsal joint, and trapezio-metacarpal joint). All participants received intra-articular HA (30 mg/2 ml) into one or more joints, as required, once a week for three consecutive weeks. The participants were then assessed at a follow-up visit occurring two weeks after the final injection to evaluate the variation in efficacy parameters compared to the baseline visit. The efficacy parameters included assessment of self-reported pain via the VAS (to assess the global level of pain in the target joint), and evaluation of motor function via the Health Assessment Questionnaire (HAQ)[48,49]. An improvement in joint pain was observed in the study population. Mean VAS for joint pain in motion significantly decreased over the study period for all joints, and a similar (but smaller) change occurred in VAS for

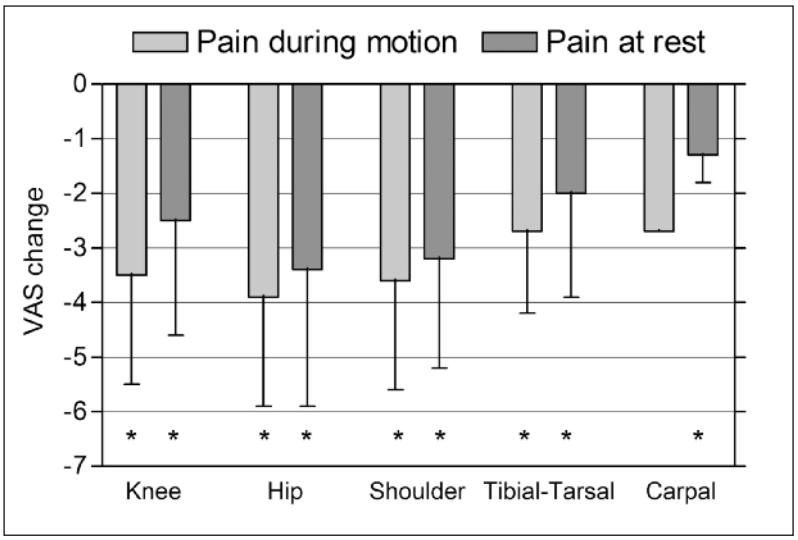

Figure 1. Mean change (± standard deviation) in joint pain during motion and at rest evaluated using the 100 mm VAS, as reported by Foti et al[47]. A decrease in VAS score indicates an improvement in pain severity. Asterisks (*) highlight significant differences ($p < 0.01$, Student's t-test) with respect to the baseline value.

pain at rest (Figure 1). As indicated by the mean absolute change in HAQ over the study period, a significant improvement in motor function was observed. Furthermore, the number of patients using NSAIDs decreased over the study period (only 4% of subjects were using drugs at the follow-up visit).

This pattern of effects also emerged from other studies (summarized in Table I) applying the same protocol of treatment (3 weekly injections), but focused on pathologies affecting specific joints.

Two studies addressed the possible use of Hyalubrix® for the treatment of trapezio-metacarpal (TMC) joint OA[50,51]. In the pilot study by Ingegnoli et al[50], involving 16 patients, 0.5 ml were administered at each ultrasound-guided intra-articular injection. A significant clinical improvement was obtained by the decrease in VAS pain already after 2 weeks of treatment and this result was maintained at week 24. Interestingly, in this study ultrasound examination of inflammatory parameters [synovial hypertrophy and Power Doppler Signal (PDS)] was also performed. PDS appeared significantly decreased after two weeks of treatment, but no significant decrease in the synovial hypertrophy was observed during the study. In the trial by Di Sante et al[51] the volume of each ultrasound-guided intra-articular injection into the TMC joint was of 1 ml, and the 31 recruited patients were evaluated before treatment and at 1, 3, and 6 months after the first injection. A statistically significant VAS score reduction was observed at 1 and 3 months, but not at 6-month follow-up.

OA of the knee was the focus of a study by Smiderle and collaborators[52,53], involving 14 subjects receiving one intra-articular injection per week for three weeks. They were evaluated at baseline and 45 days after by using subjective scores (a Numerical Rating scale for pain and the WOMAC scale) and an instrumental approach (optoelectronic digital analysis of gait), which allows the measurement of a set of biomechanical parameters[54,55]. Both approaches showed significant improvements following treatment.

A combination of subjective scores (VAS, WOMAC scale) and instrumental analysis was also used by Paoloni et al[56] to assess the effect of the three weekly intra-articular Hyalubrix® injections on 20 patients with hip OA. Prior to the commencement of treatment each participant underwent a clinical and gait analysis evaluation. Clinical evaluation was then performed at 1, 3, and 6 months after treatment ended. At the end of the follow-up period a final gait analysis was also performed. The study highlighted not only a significant improvement in terms of pain (VAS), stiffness and disability (WOMAC scale), but also a significant change in walking pattern as a consequence of the applied treatment (Figure 2).

A different treatment protocol was explored by Migliore et al[57,58] in two open-label trials on hip OA (see Table I). In the first, prospective, study[57] 120 patients received 60 mg/4 ml of Hyalubrix® every 6 months, with the possibility of an additional injection at the intervening 3-months intervals on clinical request. A significant reduc-

Table I. Clinical trials treating specific joints with intra-articular injections of Hyalubrix® (1500-2000 kDa HA, 30 mg/2 ml)

Study design and duration	Patients	Joint and pathology	Treatment	Efficacy assessment	Safety	Reference
Single-site, open-label 45 days	14 (>40 yrs; OA)	Knee OA	3 weekly injections	Significant improvement of the Numerical Rating scale for pain and in all the parameters measured with the Womac scale. Optoelectronic digital analysis of gait showed significant improvements in the biomechanical parameters of walking	Not done	Smiderle et al.[52,53]
Single-site, controlled, double-blind 12 months	109: - 55 (Hyalubrix® treated) - 54 (Platelet-rich plasma treated) (56.5 yrs)	Knee chondro-pathy or OA	3 weekly injections	Both treatments similarly improved all the evaluated parameters, with a tendency toward better results for PRP in less degenerated joints	Higher, but self-limiting, post-injective pain in PRP group.	Filardo et al.[61]
Single-site, controlled, double-blind 26 weeks	42: - 22 (Hyalubrix® treated) - 20 (Mepivacaine treated) (42-79 yrs)	Hip OA	2 monthly injections of HA (two syringes, 60 mg) or Mepivacaine 2%	HA was superior to mepivacaine in improving the Lequesne's index at 3 months and 6 months. HA was superior to mepivacaine for the VAS. In both groups NSAID intake was reduced.	2 (1 in HA, and 1 in mepivacaine group) transient local adverse events (pain at the injection site)	Migliore et al.[59]
Single-site, prospective, open-label 18 months	120 (>40 yrs)	Hip OA (>40 yrs)	An injection of HA (60 mg/4ml) every 6 months (additional injection at the intervening 3-months intervals possible)	Significant reduction of the Lequesne's index, VAS and NSAID intake at 3 months, while at 12 months 80% of patients achieved a decrease of at least 30% in symptoms. Results maintained over time through cyclical repetition of injections.	16 local, spontaneously resolved, adverse events were reported	Migliore et al.[57]
Single-site, prospective, open-label 6 months	20 (47-73 yrs)	Hip OA	3 weekly injections	Pain, as measured by VAS, significantly dropped following treatment. A significant improvement regarding stiffness and disability (Womac scale). Improved walking pattern as measured by instrumental gait analysis.	Not done	Paoloni et al.[56]
Multicenter, retrospective 48 months	176 (33-89 yrs)	Hip OA	An injection of HA (60 mg/4ml) every 6 months (additional injection at the inter-vening 3-months intervals possible)	HA treatment seems to delay surgery (Total Hip Replacement)	A mild transient pain, regressed without need of medication, was reported by 34 patients in 72 different occasions.	Migliore et al.[58]

Table I continued. Clinical trials treating specific joints with intra-articular injections of Hyalubrix® (1500-2000 kDa HA, 30 mg/2 ml)

Study design and duration	Patients	Joint and pathology	Treatment	Efficacy assessment	Safety	Reference
Single-site, open-label 24 weeks	16 (43-79 yrs)	TMC OA	3 weekly injections (0.5 ml)	Significant pain decrease (as measured by VAS) after 2 weeks of treatment and this result is maintained at week 24. Power Doppler signal decreased at 2 weeks, but this result was not maintained at week 24. No significant decrease in the synovial hypertrophy.	No adverse events reported	Ingegnoli et al.[50]
Single-site, open-label 6 months	31	TMC OA	3 weekly injections (1 ml)	A statistically significant VAS score improvement was observed at 1 and 3 months following treatment, but not at 6 months follow-up. No improvement of the Duruöz Hand Index was observed	No adverse events reported	Di Sante et al.[51]
Multicenter, controlled, open-label 60 days	100: - 51 (Hyalubrix® treated) - 49 (standard treatment) (40-50 yrs)	Knee (arthroscopic meniscectomy)	Single injection in the final phase of arthroscopy	Significant improvements with HA vs. standard treatment for relief of pain during activity and at rest, and for general joint mobility	No difference between groups in terms of reported transient local adverse events (5 in the HA group, 4 in thecontrol group)	Priano and Guelfi[63]

tion of algofunctional indexes (Lequesne's index) was demonstrated three months after the first injection, suggesting that this regimen of treatment can provide similar results to those obtained with the standard protocol. Furthermore, therapeutical benefits were maintained over time through cyclical repetition of injections. The second study[58] was a multicenter, retrospective, study in which it was assessed whether 176 patients suffering from hip OA were candidate for total hip replacement (THR) according to clinical data collected at the first visit for ultrasound-guided intra-articular injections of Hyalubrix® (60 mg/4 ml). At 24 months 90% of the patients did not undergo THR, and at 48 months 82% of the study population treated with intra-articular HA avoided THR. Thus, although further studies are necessary to confirm these data and to identify outcome predictors, this result suggested that hip viscosupplementation with HA may delay the need for surgical intervention.

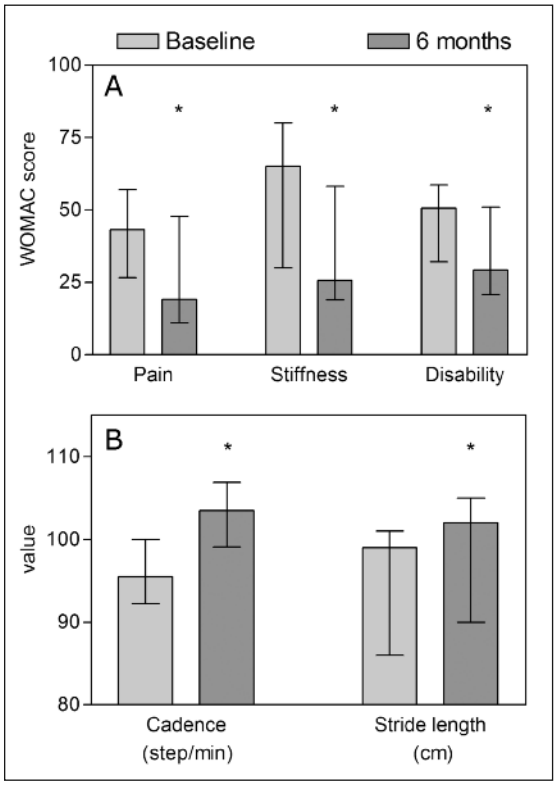

Figure 2. Main results reported by Paoloni et al.[56] in their study on hip OA. ***A,*** WOMAC scores at baseline and 6 months following a treatment cycle of 3 weekly intra-articular injections of Hyalubrix®. ***B,*** Spatio-temporal characteristics of gait at pre- and post-treatment evaluations. Data are median values with error bars representing 25th and 75th percentiles. Asterisks (*) indicate significant differences ($p < 0.05$, paired t-test or Wilcoxon test) with respect to the baseline value.

Comparative trials

In a double-blind, controlled, trial comparing Hyalubrix® to a local anesthetic[59] HA (60 mg/4 ml) or mepivacaine (2%) was administered twice (once a month) to 42 patients suffering from hip OA. Efficacy measurements included the Lequesne's algofunctional index, a VAS for pain, concomitant use of NSAIDs, and patient and physician global evaluation. Both treatments significantly improved the Lequesne's index versus baseline at 3 and 6 months, HA treatment resulting significantly superior to mepivacaine (Figure 3). The same result was found on pain intensity, as measured by VAS. The NSAIDs intake was reduced in both groups after three months (49% with HA, 25% with mepivacaine), with no statistically significant differences between the two treatments. The same trend was observed for what it concerns the global assessment by patients and physician.

Platelet Rich Plasma (PRP), a blood-derived product rich in growth factors, is a promising treatment for cartilage defects[60]. A randomized, double-blind, prospective study by Filardo et al[61] compared this treatment to Hyalubrix®. 109 patients affected by knee degenerative pathology underwent a cycle of three weekly intra-articular injections of HA or PRP and were evaluated basally, and at 2, 6 and 12 months of follow-up by orthopaedic scores (IKDC, KOOS, EQ-VAS) for general status and Tegner scale[62] scoring the activity level. Range of motion and knee circumference changes were also measured over time. Although in this study HA was used as the reference, control, treatment, the trial provided interesting information on the high-MW HA therapy. In fact, at the follow-up evaluations, both groups presented a significant clinical improvement but the comparison between the two groups showed a not statistically significant difference in all scores evaluated. A trend favorable for the PRP group was only found in patients with low grade articular degeneration (Kellgren-Lawrence score up to 2).

A comparative study exploring the possible use of Hyalubrix® after knee arthroscopy was performed by Priano and Guelfi[63]. In this study 100 patients who underwent arthroscopic meniscectomy were divided into two treatment groups: 51 were treated with Hyalubrix® injected into the joint cavity after completion of the procedure, and 49 underwent conventional post-surgical therapy, without the administration of HA. When compared to the standard treatment, pain on walking, at rest, during activity and at pressure always decreased to a greater extent in the HA-treated group, who reported also a higher level of joint mobility and functional evaluation according to the Lysholm scale scoring[62]. Thus, this study suggested that the use of high-MW HA in knee arthroscopy is associated with a significantly more favourable post-operative clinical outcome.

Almost all the above described trials also collected data on the safety and tolerability of the therapy with Hyalubrix®. The pattern of reported adverse events (mostly of minor clinical significance) is in line with the one tipically observed for HA products[64], namely transient local reactions such as reddening, itching or pain at the injection site. With reference to the two large-scale studies

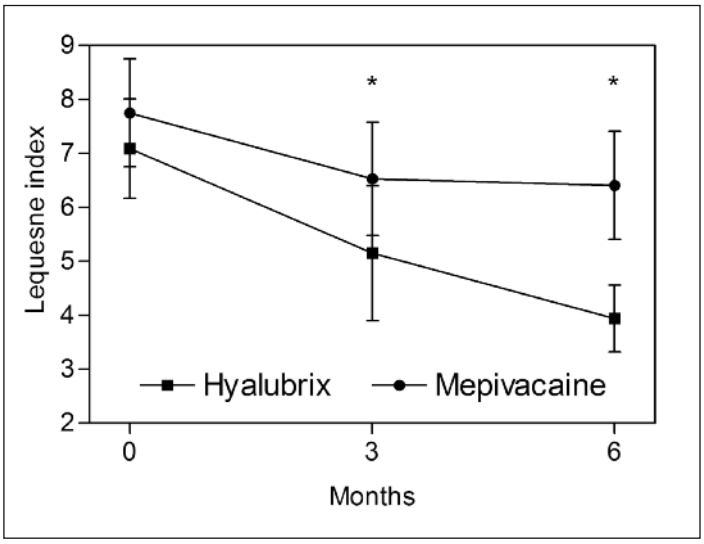

Figure 3. Results concerning the primary endpoint (Lequesne index) in the study by Migliore et al [59], comparing Hyalubrix® to a local anesthetic (mepivacaine) in hip OA. HA (60 mg/4 ml) or mepivacaine (2%) was administered twice (once a month). Both treatments significantly reduced the Lequesne index with respect to the baseline value, but the reduction, at both 3 and 6 months follow-up, was significantly higher (* = $p < 0.05$, Wilcoxon test) in the HA group when compared to the mepivacaine one.

here reviewed, the incidence of adverse events was of 0.5% in the trial by Schieb[46] and of 0.8% in the study by Foti et al[47]. Thus, the estimated risk recorded equated to approximately three adverse events per 1000 vials injected. In the controlled study by Migliore et al[59] no difference in the incidence of adverse reactions was observed between the two treatment groups, suggesting that they were not so much related to the injected substance, but rather to the injection procedure.

Discussion

Osteoarthritis is a common degenerative musculoskeletal disease, occurring in approximately 10% of people aged 55 years or older[65], and responsible for considerable clinical and economic burden as a result of reduced quality of life, increased use of health care resources and loss of productivity[66]. It can affect any synovial joint, but it is mainly seen in hands and weight-bearing joints (knee, hip), and is characterized by loss of articular cartilage and subsequent remodeling of periarticular bone[67]. Patients typically present with pain, inflammation, and/or stiffness occurring in one or more joints, which fluctuates in intensity and localization over time. To date, there is no a definitive cure for OA. According to the EULAR recommendations[68,69], the management of the pathology requires a combination of non-pharmacological (education, weight reduction, exercise and mechanical support) and pharmacological treatment modalities.

Symptomatic pharmacotherapy for OA primarily consisted of non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular injections of corticosteroid. However, the associated side-effects of these agents generated interest in developing alternative treatment modalities.

In this respect, since osteoarticular disease reduces the rheological properties of synovial fluid in the various joints affected, increasing the susceptibility of the articular cartilage to damage, a therapeutic strategy, called *viscosupplementation*, was proposed in which intra-articular injections of a suitable material are used to restore the viscoelastic properties of the synovial fluid. Solutions of high-MW native HA, which is the main component of the synovial fluid, were a natural choice to reach this goal, but HA-derived materials, engineered to achieve greater elastoviscosity and intra-articular residence time, were also developed. They include, for instance, hylans[70] and hyadd[71]. A Cochrane meta-analysis[72] of 76 trials showed that viscosupplementation is effective in OA of the knee with beneficial effects on pain, function and patient global assessment. More recent meta-analyses confirmed this finding[73]. However, the analysts often reported that there was marked variability between different preparations on different outcome parameters. In the present paper available data on Hyalubrix®, a 1.5% formulation of natural HA with MW in the range 1500-2000 kDa, were specifically reviewed.

Physicochemical studies on the HA fraction used in such a formulation generally supported its use as a viscosupplement, since it is characterized by rheological[38], lubricating[39], and hydrodynamic[42] properties quite similar to those exhibited by the SF. Furthermore, HA in this MW range when intra-articularly injected established a limited interaction with the cells of the surrounding tissues. Thus, Hyalubrix® appeared well suited to exploit a mechanism of action of biomechanical type, consistent with the viscosupplementation concept.

Although the available clinical studies here analyzed addressed the treatment of different joints (in particular knee, hip, TMC), all of them shared the conclusion that the intra-articular administration of Hyalubrix® reduced pain, improved mobility, and increased the quality of life in patients with OA. A controlled study[59] also showed a significantly better performance of the HA therapy when compared to the administration of a local anesthetic (mepivacaine). Interestingly, for what it concerns knee and hip joints these results were assessed not only by well recognized, subjective, scales and scores (as, for instance VAS, WOMAC, Lequesne's index), but in some trials[52,53,56] they were also confirmed through an instrumental approach (gait analysis) directly measuring the biomechanical characteristics of the walking pattern. The treatment protocol followed in the majority of the available studies involved three intra-articular injections once a week for three consecutive weeks, and the beneficial effects were observed quite early, sometimes after the second injection[50]. Migliore and collaborators in their studies on hip OA[57-59] investigated a different treatment protocol based on a single injection of a higher volume of Hyalubrix® (60mg/4ml), and demonstrated its efficacy in terms of symptomatic relief. Although limited to hip joint, the results of this experience are intriguing, because this treatment strategy allowed to repeat injections at time intervals of months, suggesting a way to maintain the beneficial effect over time.

Anyway, beneficial effects lasting weeks or some month were also reported by many of the studies here considered after the standard treatment cycle of three weekly injections (see Table I). This finding cannot be fully understood on the basis of the pharmacokinetics characteristics of 1500-2000 kDa HA. In fact, it is characterized by a residence time[44] significantly longer than the one exhibited by HA of lower MW, but still limited to about 120 hours. It has to be considered, however, that in addition to the temporary restoration of SF lubrication and viscoelasticity, natural HA may have a direct effect on reducing joint nociceptor activity, in part due to the role of hyaluronan as a mechanical filter that is associated to its rheological properties, but also to a chemical interaction with inflammatory mediators that reduce their sensitising effect on the nociceptor terminals of the joint tissues[74,75]. Thus, the overall reduction of joint inflammation and nociceptive sensitivity could lead to a sustained beneficial symptomatic effect of the therapy. It could also be surmised that the injected high-MW HA could provide, as a result of its depolymerization in the synovial joint, HA chains with MW suitable (500-1000 kDa) to induce viscoinduction effects (i.e. receptor-mediated) at the level of synovial membrane and cartilage. Although more deep and specific investigation on this aspect would be needed, a few data on Hyalubrix® seem to challenge this hypothesis. In fact, the observed pattern of degradation fragments generated from an injected HA of about 2000 kDa does not seem to include a significant amount of 500-1000 kDa chains[44]. Consistently, in one of the trials here reviewed[50] no reduction of the synovial hypertrophy was observed following treatment with Hyalubrix®, whereas improvements at tissue level are well documented when HA with MW in the range 500-1000 kDa is used for intra-articular therapy[24,76,77]. Based on these findings, it could be proposed that high MW HA and HA with MW in the range 500-1000 kDa represent two distinct strategies for the management of the osteoarthritic patient, the latter being more suitable in patients with low grade articular degeneration, where some tissue response and possibly repair can still be stimulated. The data from the here reviewed study by Filardo et al[61] indirectly support this view.

Being based on native HA, the biomechanical performance of Hyalubrix® also differs from the one provided by HA derivatives for intra-articular therapy specifically engineered by chemical modification to achieve greater viscoelastic properties and higher dwell-time. It has to be taken into account, however, that in contrast with native HA, HA derivatives appear associated with a higher incidence of adverse events (probably linked to the higher immunogenicity of these materials[78]). Goldberg and Coutts[79] report an incidence of 8-27% of acute local reactions with the use of hylans, whereas from the body of studies here considered the incidence of adverse events with Hyalubrix® is 0.5-0.8%, mostly of minor clinical significance.

Conclusions

Finally, the data from some of the studies here analyzed support the possibility that viscosupplementation with high MW HA could be of benefit not only in OA, but also in conditions like traumatic arthropaty[46] and to get a more favourable outcome following arthroscopy[63]. Thus, the overall available data outline for Hyalubrix® viscosupplementation the profile of a valuable tool for the symptomatic management of patients with different forms of joint arthropathy.

Acknowledgements

The author thanks dr. Piera Parenzan and dr. Pierangelo Bellato (Fidia Farmaceutici SpA, Italy) for bibliographic support.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Laurent TC, Fraser JRE. Hyaluronan. FASEB J 1992; 6: 2397-2404.
2) Fraser JRE, Laurent TC, Laurent UBG. Hyaluronan: Its nature, distribution, functions and turnover. J Intern Med 1997; 242: 27-33.
3) Balazs EA, Watson D, Duff IF, Roseman S. Hyaluronic acid in synovial fluid. I. Molecular parameters of hyaluronic acid in normal and arthritic human fluids. Arthritis Rheum 1967; 10: 357-376.
4) Bothner H, Wik O. Rheology of hyaluronate. Acta Otolaryngol (Stockh) 1987; Suppl 442: 25-30.
5) Balazs EA, Denlinger JL. Sodium hyaluronate and joint function. Equine Vet Sci 1985; 5: 217-228.
6) Cullis-Hill D, Ghosh P. The role of hyaluronic acid in joint stability--a hypothesis for hip displasia and allied disorders Med Hypotheses 1987; 23: 171-185.
7) Ogston AG, Preston BN. The exclusion of protein by hyaluronic acid. Measurement by light scattering. J Biol Chem 1966; 241: 17-19.

8) Levick JR. Synovial fluid hydraulics. Sci Med 1996; 3: 52-62.
9) McDonald JN, Levick JR. Hyaluronan reduces fluid escape rate from rabbit knee joints disparately from its effect on fluidity. Exp Physiol 1994; 79: 103-106.
10) Scott D, Coleman PJ, Mason RM, Levick JR. Concentration-dependence of interstitial flow buffering by hyaluronan in synovial joints. Microvasc Res 2000; 59: 345-353.
11) Fakhari A, Berkland C. Application and emerging trends of hyaluronic acid in tissue engineering, as a dermal filler and in osteoarthritis treatment. Acta Biomater 2013; 9: 7081-7092.
12) Dahl LB, Dahl IMS, Engstrom-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.
13) Greenwald RA. Oxygen radicals, inflammation, and arthritis - pathophysiological considerations and implications for treatment. Semin Arthritis Rheum 1991; 20: 219-240.
14) Stephens RW, Ghosh P, Taylor TKF, Gale CA, Swann JC, Robinson RG, Webb J. The origins and relative distribution of polysaccharidases in rheumatoid and oesteoarthritic fluids. J Rheumatol 1975; 2: 393-400.
15) Stair-Nawy S, Csoka AB, Stern R. Hyaluronidase expression in human skin fibroblasts. Biochem Biophys Res Commun 1999; 266: 268-273.
16) Balazs EA, Denlinger JL. Viscosupplementation: A new concept in the treatment of osteoarthritis. J Rheumatol 1993; 20: 3-9.
17) Rydell N, Balazs EA. Effect of intra-articular injection of hyaluronic acid on the clinical symptoms of osteoarthritis and on granulation tissue formation. Clin Orthop 1971; 80: 25-32.
18) Balazs EA, Band P. Hyaluronic acid - its structure and use. Cosmet Toiletries 1984; 99: 65-72.
19) Balasz EA. Viscosupplementation for treatment of osteoarthritis: from initial discovery to current status and results. Surg Technol Int 2004; 12: 278-89.
20) Peyron JG, Balazs EA. Preliminary clinical assessment of Na-hyaluronate injection into human arthritic joints. Pathol Biol 1974; 22: 731-736.
21) Peyron JG. A new approach to the treatment of osteoarthritis: viscosupplementation. Osteoarthritis Cartilage 1993; 1: 85-87.
22) Dougados M, Nguyen M, Listrat V, Amor B. High molecular weight sodium hyaluronate (hyalectin) in osteoarthritis of the knee: a 1 year placebo controlled trial. Osteoarthr Cartil 1993; 1: 97-103.
23) Altman RD, Moskowitz R. Intraarticular sodium hyaluronate (Hyalgan) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. Hyalgan Study Group. J Rheumatol 1998; 25: 2203-2212.
24) Listrat V, Ayral X, Patarnello F, Bonvarlet JP, Simonnet J, Amor B, Dougados M. Arthroscopic evaluation of potential structure modifying activity of hyaluronan (Hyalgan) in osteoarthritis of the knee. Osteoarthr Cartil 1997; 5: 153-160.
25) Frizziero L, Govoni E, Bacchini P. Intra-articular hyaluronic acid in the treatment of osteoarthritis of the knee: clinical and morphological study. Clin Exp Rheumatol 1998; 16: 441-449.
26) Fraser JRE, Brown TJ, Cahill TNP, Laurent TC, Laurent UBG. The turnover of hyaluronan in synovial joints (abstract). Immunol Cell Biol 1996; 74: A10.
27) Laurent UBG, Fraser JRE, Engström-Laurent A, Reed RK, Dahl LB, Laurent TC. Catabolism of hyaluronan in the knee joint of the rabbit. Matrix 1992; 12: 130-136.
28) Sakamoto T, Mizono S, Miyazaki K, Yamaguchi T, Toyoshima H, Namiki O. Biological fate of sodium hyaluronate (SPH): Studies on the distribution, metabolism, and excretion of 14C-SPH in rabbits after intra-articular administration. Pharmacometrics 1984; 28: 375-87.
29) Ghosh P, Guidolin D. Potential mechanism of action of intra-articular hyaluronan therapy in osteoarthritis: are the effects molecular weight dependent ? Semin Arthritis Rheum 2002; 32:10-37.
30) Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr Opin Cell Biol 2000; 12: 581-586.
31) Moreland LW. Intra-articular hyaluronan (hyaluronic acid) and hylans for the treatment of osteoarthritis: mechanisms of action. Arthritis Res Ther 2003; 5: 54-67.
32) Ohkawara Y, Tamura G, Iwasaki T, Tanaka A, Kikuchi T, Shirato K. Activation and transforming growth factor beta production in eosinophils by hyaluronan. Am J Respir Cell Mol Biol 2000; 23: 444-451.
33) Yang C, Cao M, Liu H, He Y, Xu J, Du Y, Liu Y, Wang W, Cui L, Hu J, Gao F. The high and low molecular weight forms of hyaluronan have distinct effects on CD44 clustering. J Biol Chem 2012; 287: 43094-43107.
34) Smith MM, Ghosh P. The synthesis of hyaluronic acid by human synovial fibroblasts is influenced by the nature of the hyaluronate in the extracellular environment. Rheumatol Int 1987; 7: 113-122.
35) Gigante A, Callegari L. The role of intra-articular hyaluronan (Sinovial) in the treatment of osteoarthritis. Rheumatol Int 2011; 31: 427-444.
36) HYAP15 (Hyalubrix®). Clinical investigator's brochure. 2004; Padova: Fidia Farmaceutici SpA.
37) Gibbs D, Merrill E, Smith K. Rheology of hyaluronic acid. Biopolymers 1968; 6:777-791.
38) Mazzucco D, McKinley G, Scott RD, Spector M. Rheology of joint fluid in total knee arthroplasty patients. J Orthop Res 2002; 20: 1157-1163.
39) Yokobori AT, Kawaharada T, Sasaki S, Watanabe S, Fang SH, Komatsu S, Hayashi T. Mechanical test method on the estimation of the lubricant performance by Hyaluronic Acid. Biomed Mat Eng 1995; 5: 117-124.
40) Pasquali Ronchetti I, Quaglino D, Mori G, Bacchelli B, Ghosh P. Hyaluronan-phospholipid interactions. J Struct Biol 1997; 120: 1-10.
41) Nitzan DW, Nitzan U, Dan P, Yedgar S. The role of hyaluronic acid in protecting surface-active phospholipids from lysis by exogenous phospholipase A2. Rheumatology 2001; 40: 336-340.

42) Coleman PJ, Scott D, Mason RM, Levick JR. Role of hyaluronan chain length in buffering interstitial flow across synovium in rabbits. J Physiol 2000; 526.2: 425-434.

43) Asari A, Miyauchi S, Matsuzaka S, Ito T, Kominami E, Uchiyama Y. Molecular weight-dependent effects of hyaluronate on the arthritic synovium. Arch Histol Cytol 1998; 61: 125-135.

44) Komatsu S, Iwata H, Nabeshima T. Studies on the kinetics, metabolism and re-utilization after intra-articular administration of hyaluronan to rabbits. Arzneim Forsch 1999; 49: 427-433.

45) Fraser JR, Kimpton WG, Pierscionek BK, Cahill TNP. The kinetics of hyaluronan in normal and acutely inflamed synovial joints: observations with experimental arthritis in sheep. Semin Arthritis Rheum 1993; 22(S1): 9-17.

46) Schieb F. Intraartikulär injizierte Hyaluronsäure bei arthropathien. Arthritis Rheum 2003; 6: 338-340.

47) Foti C, Cisari C, Carda S, Giordan N, Rocco A, Frizziero A, Della Bella G. A prospective observational study of the clinical efficacy and safety of intra-articular sodium hyaluronate (MW 1500-2000 kDa; Hyalubrix®) in synovial joints with osteoarthritis. Eur J Phys Rehabil Med 2011; 47: 1-9.

48) Ranza R, Marchesoni A, Calori G, Bianchi G, Braga M, Canazza S, Canesi B, Fumagalli M, Mastaglio C, Mathieu A. The italian version of the Functional Disability Index of the Health Assessment Questionnaire. A reliable instrument for multicenter studies on rheumatoid arthritis. Clin Exp Rheumatol 1993; 11: 123-128.

49) Bruce B, Fries J. The Stanford health assessment questionnaire (HAQ): a review of its history, issues, progress, and documentation. J Rheumatol 2003; 30: 167-178.

50) Ingegnoli F, Soldi A, Meroni PL. Power Doppler sonography and clinical monitoring for hyaluronic acid treatment of rhizarthrosis: a pilot study. J Hand Microsurg 2011; 3: 51-54.

51) Di Sante L, Cacchio A, Scettri P, Paoloni M, Ioppolo F, Santilli V. Ultrasound-guided procedure for the treatment of trapeziometacarpal osteoarthritis. Clin Rheumatol 2011; 30: 1195-1200.

52) Smiderle C, Scapin M, Baldo M, Ronconi L, Marcolin G, Villaminar R. Gait analysis of changes in clinical and biomechanical parameters in osteoarthritis knee patients after intraarticular infiltration with high molecular weight hyaluronan. Eur Med Phys 2007; 43: 1-3.

53) Smiderle C, Scapin M, Ronconi L, Baldo M, Villaminar R. Changes in clinical and biomechanical parameters in osteoarthritis knee patients after intra-articular infiltration with hyaluronic acid h.m.w. In: Proceedings of the 17th annual meeting of the European Society of Movement Analysis for adults and children. Turin: Edizioni Minerva Medica, 2008.

54) Gok H, Ergin S, Yavuzer G. Kinetic and Kinematic characteristics of gait in patients with medial knee arthrosis. Acta Orthop Scand 2002; 73: 647-652.

55) Chen CPC, Chen MJL, Pei YC, Lew HL, Wong PY, Tang SFT. Sagittal plane loading response during gait in different age groups and in people with knee osteoarthritis. Am J Phis Med Rehabil 2003; 82: 307-312.

56) Paoloni M, Di Sante L, Dimaggio M, Bernetti A, Mangone M, Di Renzo S, Santilli V. Kinematic and kinetic modifications in walking pattern of hip osteoarthritis patients induced by intra-articular injections of hyaluronic acid. Clin Biomech 2012; 27: 661-665.

57) Migliore A, Massafra U, Bizzi E, Laganà B, Valentina G, Piscitelli P, Granata M, Tormenta S. Intra-articular injection of hyaluronic acid (MW1500-2000 kDa; HyalOne®) in symptomatic osteoarthritis of the hip: a prospective cohort study. Arch Orthop Trauma Surg 2011; 131: 1677-1685.

58) Migliore A, Bella A, Bisignani M, Calderaro M, De Amicis D, Logroscino G, Mariottini F, Moreschini O, Massafra U, Bizzi E, Laganà B, Piscitelli P, Tormenta S. Total hip replacement rate in a cohort of patients affected by symptomatic hip osteoarthritis following intra-articular sodium hyaluronate (MW 1500-2000 kDa). ORTOBRIX study. Clin Rheumatol 2012; 31: 1187-1196.

59) Migliore A, Massafra U, Bizzi E, Vacca F, Martin-Martin S, Granata M, Alimonti A, Tormenta S. Comparative, double-blind, controlled study of intra-articular hyaluronic acid (Hyalubrix®) injections versus local anesthetic in osteoarthritis of the hip. Arthritis Res Ther 2009; 11: R183.

60) Filardo G, Kon E. PRP: more words than facts. Knee Surg Sports Traumatol Arthrosc 2012; 20:1655-1656.

61) Filardo G, Kon E, Di Martino A, Di Matteo B, Merli ML, Cenacchi AR, Fornasari PM, Marcacci M. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. BMC Musculoskelet Disord 2012; 13: 229.

62) Tegner Y, Lysholm J. Rating systems in the evaluation of knee ligament injuries. Clin Orthop 1985; 198: 43-49.

63) Priano F, Guelfi M. Efficacy of intra-articular hyaluronic acid (Hyalubrix®) in arthroscopy. Artroscopia 2007; 8: 1-12.

64) Hamburger MI, Lakhanpal S, Mooar PA, Oster D. Intra-articular hyaluronans:a review of product-specific safety profiles. Semin Arthritis Rheum 2003; 32: 296-309.

65) Arrich J, Piribauer F, Mad P, Schmid D, Klaushofer K, Mullner M. Intra-articular hyaluronic acid for the treatment of the knee: systematic review and meta-analysis. CMAJ 2005; 172: 1039-1043.

66) Carr AJ. Beyond disability: measuring the social and personal consequences of osteoarthritis. Osteoarthr Cartil 1999; 7: 230-238.

67) National Institute of Clinical Excellence. OSTEOARTHRITIS national clinical guideline for care and management in adults. 2009; http://www.nice.org.uk/nicemedia/pdf/CG059FullGuideline.pdf. Last accessed: July 5, 2013.

68) Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, Gunther K, Hauselmann H, Herrero-Beaumont G, Kaklamanis P, Lohmander S, Leeb B, Lequesne M, Mazieres B, Martin-Mola E,

Pavelka K, Pendleton A, Punzi L, Serni U, Swoboda B, Verbruggen G, Zimmerman-Gorska I, Dougados M. EULAR recommendations 2003: an evidence based approach to the management of knee osteoarthritis. Report of a task force of the Standing Committee for International Clinical Studies Including Therapeutic Trials (ESCISIT). Ann Rheum Dis 2003; 62: 1145-1155.

69) Zhang W, Doherty M, Arden N, Bannwarth B, Bijlsma J, Gunther KP, Hauselmann HJ, Herrero-Beaumont G, Jordan K, Kaklamanis P, Leeb B, Lequesne M, Lohmander S, Mazieres B, Martin-Mola E, Pavelka K, Pendleton A, Punzi L, Swoboda B, Varatojo R, Verbruggen G, Zimmermann-Gorska I, Dougados M. EULAR evidence based recommendations for the management of hip osteoarthritis: report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis 2005; 64: 669-681.

70) Migliore A, Giovannangeli F, Granata M, Laganà B. Hylan g-f 20: review of its safety and efficacy in the management of joint pain in osteoarthritis. Clin Med Insights Arthritis Musculoskelet Disord 2010; 3: 55-68.

71) Mainil-Varlet P, Schiavinato A, Ganster MM. Efficacy evaluation of a new hyaluronan derivative HYADD 4G to maintain cartilage integrity in a rabbit model of osteoarthritis. Cartilage 2013; 4: 28-41.

72) Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev 2006; 2: CD005321.

73) Bannuru RR, Natov NS, Dasi UR, Schmid CH, McAlindon TE. Therapeutic trajectory following intra-articular hyaluronic acid injection in knee osteoarthritis—meta-analysis. Osteoarthritis Cartilage 2011; 19: 611-619.

74) Pozo MA, Balazs EA, Belmonte C. Reduction of sensory responses to passive movements of inflamed knee joints by hylan, a hyaluronan derivative. Exp Brain Res 1997; 116: 3-9.

75) Gomis A, Miralles A, Schmidt RF, Belmonte C. Intraarticular injections of hyaluronan solutions of different elastoviscosity reduce nociceptive nerve activity in a model of osteoarthritic knee joint of the guinea pig. Osteoarthritis Cartilage 2009; 17: 798-804.

76) Pasquali Ronchetti I, Guerra D, Taparelli F, Boraldi F, Bergamini G, Mori G, Zizzi F, Frizziero L. Morphological analysis of knee synovial membrane biopsies from a randomized controlled clinical study comparing the effect of sodium hyaluronate (Hyalgan) and methylprednisolone acetate (Depomedrol) in osteoarthritis. Rheumatology 2001; 40: 158-169.

77) Guidolin D, Pasquali Ronchetti I, Lini E, Guerra D, Frizziero L. Morphological analysis of articular cartilage biopsies from a randomized, clinical study comparing the effect of 500-730 kDa sodium hyaluronate (Hyalgan) and methylprednisolone acetate on primary osteoarthritis of the knee. Osteoarthr Cartil 2001; 9: 371-381.

78) Dragomir CL, Scott JL, Perino G, Adler R, Fealy S, Goldring MB. Acute inflammation with induction of anaphylatoxin C5a and terminal complement complex C5b-9 associated with multiple intra-articular injections of hylan G-F20: a case report. Osteoarthritis Cartilage 2012; 20: 791-795.

79) Goldberg VM, Coutts RD. Pseudoseptic reactions to hylan viscosupplementation: diagnosis and treatment. Clin Orthop Relat Res 2004; 419: 130-137.