Efficacy and absorption of hyaluronic acid and N-acetyl-D-glucosamine for the treatment of osteoarthritis: a review

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ABSTRACT

Osteo-articular diseases affect many elderly people, resulting in worse quality of life and a substantial public health cost. Osteoarthritis, inflammatory articular diseases and conditions associated with cartilage disruption are the most frequently diagnosed. Non-steroidal anti-inflammatory drugs, glucocorticoids and physiotherapy are used to treat affected patients, while some nutraceutical products containing chondroprotective and osteotropic substances have been shown to improve their signs and symptoms. However, the true absorption and efficacy of these substances in humans is largely unknown. The absorption of hyaluronans and chondroitin sulfate is likely negatively affected by their high molecular weight. Nevertheless, many published papers have reported significant improvements in symptoms and articular functionality in patients taking these compounds. This paper attempts to clarify the apparent dichotomy between absorption and efficacy, and compare the clinical evidence for the bioavailability of hyaluronic acid with that of its precursor N-acetyl glucosamine.

Introduction

Osteoarthritis (OA) is a degenerative joint disease which occurs when the cartilage, or cushion, in the joint is damaged. It is the most common form of arthropathic disorder and mostly affects the elderly [1, 2], leading to pain, stiffness and limited mobility. OA shows reduced hyaluronic acid (HA) concentration [3], cartilage disruption [4], local inflammation of tendons, decreased synovial fluid [4, 5], osteophyte formation and muscle weakness [4].

The aetiology of OA is complex and multifactorial, involving different biochemical pathways and cellular changes [6]. However, disease initiation and progression are not clearly understood. Pharmacological treatment of OA includes acetaminophens [7], non-steroidal anti-inflammatory drugs (NSAIDs) [7] and corticosteroids [8]. In recent years, increasing interest has also been focused on nutraceutical preparations, which include hyaluronic acid (HA) [9, 10], its precursor N-acetyl-D-glucosamine (NAG) [10, 11] and

chondroitin sulphate (CS). Investigations have demonstrated their supportive role and efficacy in counteracting the signs and symptoms of OA [9], but the available data regarding their mechanism of action and extent of absorption after oral administration are unclear. The aim of this paper is to focus on clinical investigations related to HA and its precursor NAG in order to elucidate current knowledge on this topic.

Hyaluronic acid

First discovered in 1934 by Meyer *et al* [12], HA is a non-sulphated glycosaminoglycan which occurs naturally in a number of body tissues. HA is a polysaccharide composed of linear repeating disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic β (1→3) bond [13]. The molecular weight of HA ranges from 20 to 4,000 kDa [14] and polymers can adopt different configurations according to their molecular weight, salt concentration, pH and associated cations [15]. At physiological pH, HA occurs in the salt form, hyaluronate, generally sodium hyaluronate [16]. HA is a major component of extracellular matrix (ECM) and peri-cellular matrix, and is the main molecule in the gel-like structure found in soft connective tissue including the skin, the umbilical cord, synovial fluid and vitreous humor, as well as in the brain, heart valves and lungs [17].

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The main property of HA is its ability to bind water, resulting in strong tissue hydration [18]. HA is mainly known for providing joint lubrication [19], tissue viscoelasticity [20] and dermal tissue hydration [21]; it is also involved in immune system responses [22, 23], wound healing processes [24] and the formation of connective tissue in blood vessels [25]. Furthermore, HA interacts with a group of matrix proteins, called hyaladherins (HYA), to modulate cellular activity, cell migration, differentiation and adhesion [26].

HA is synthetized by membrane-bound enzymes, called HA synthases (HAS). Three different enzymes are involved in HA synthesis, HAS 1, 2 and 3, according to HA chain length [27]. Depending on the molecular weight, HA polymers exhibit different activities [28].

HA is extensively used in the healthcare, cosmetic and pharmaceutical industries and, because of its physiological role in synovial fluid, is proposed as a supplement [9, 29] or alternatively as sterile jellified fluid for injection into the synovial space to relieve the signs and symptoms of OA. It is speculated that the main effects of HA in OA management include restoration of synovial viscoelasticity, intervention in cartilage biosynthesis and degradation processes [9], and an anti-inflammatory and analgesic effect [9].

HA oral absorption in humans is still the subject of investigation. The mechanism of intestinal internalization has still to be elucidated, with findings being controversial so far. A series of studies using radiolabelled HA suggests the possible kinetics of absorption and distribution in tissue. It has been demonstrated that in rats oral HA is absorbed and accumulates in all connective tissue, persisting for 48 hours [30]. This finding has been confirmed in rats by Oe *et al* who showed that 90% of orally administered HA is absorbed in the intestine and distributed in the skin [31]. Conversely, Laznicek *et al* reported that only a low level of HA is detected in the central compartment after oral consumption [32]. A recent study in rats speculated that HA is metabolised in the cecum by bacteria before the oligosaccharides formed are absorbed and migrate though the tissues [33]. However, orally administered HA has limitations due to its high molecular weight and short half-life [34], and clear conclusions have not yet been reached. It is suggested that polymers with different molecular weights have different bioavailability profiles. Hisada *et al* demonstrated that low-molecular weight HA permeates through the Caco-2 cell model [35] and absorption is inversely correlated with chain size [35]. Studies have indicated that HA modification, such as acetylation [36] or producing a complex with phospholipids [37], could increase HA bioavailability [36, 37].

Clinical evidence for hyaluronans

The chondroprotective role of HA and its precursor NAG is well established both in animal models and in humans [9, 10]. Many randomized, double-blind, placebo-controlled clinical trials have been carried out in the recent years, mostly related to knee OA.

In 2008, a total of 20 subjects with knee OA symptoms for more than 6 months were enrolled in a randomized, double-blind, controlled trial. Ten subjects were administered 40 mg of HA from a natural extract of chicken combs for 2 months and were compared with a control group taking placebo. After treatment, a statistically significant improvement according to the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was seen in physical function and overall symptom scores in the HA group. Moreover, quality of life as measured by the Short Form-36 showed higher scores in the HA group compared with baseline in some items, such as bodily pain and the physical component summary [38]. This result is in line with the findings of a placebo-controlled, double-blind trial conducted by Sato and Iwaso [39]. Their study showed that a daily intake of 200 mg of HA for 2 months provided relief in knee OA according to WOMAC [39]. The same authors examined the impact of oral HA supplementation using the Japanese Knee Osteoarthritis Measure (JKOM) score, which showed that 2-month treatment with 240 mg of HA improved quality of life [40].

In 2010, a randomized, double-blind, placebo-controlled trial showed that 16 weeks of treatment with 60 mg of HA improved Japanese Orthopaedic Association (YOA) response criteria scores. 'Pain/walking function', 'pain/step-up and -down function' and 'aggregate total symptoms' scores were improved in the treated group compared with control subjects [41]. Furthermore, collagen synthesis was positively affected by HA as shown by collagen metabolism biomarkers [41].

Tashiro *et al* [42] studied HA oral supplementation for 12 months in a double-blind, placebo-controlled study. Sixty patients over 50 years of age with symptomatic knee OA were randomly given 200 mg of HA daily together with leg strengthening exercises. Symptom variation was monitored using the JKOM score. HA supplementation improved OA symptoms but seemed to be more effective among subjects below 70 years of age. Furthermore, better relief was seen early in the study at the 2nd and 4th months rather than at the 6th and 12th months when results were not significant [42].

Martinez-Puig *et al* evaluated the efficacy of HA admin-

istered in a yoghurt matrix. Forty subjects with mild joint pain were enrolled in a randomized, double-blind, placebo-controlled intervention trial. The study evaluated muscle strength using an isokinetic dynamometer. The authors concluded that HA supplementation improves the ability of the knee to bend and stretch [43]. Solà et al confirmed this result by showing yoghurt supplemented with rooster comb extract rich in HA and taken for 3 months improved muscle power in individuals affected by mild knee discomfort [44]. In 2015, Nelson et al [45] administered an oral formulation containing 56 mg of HA for 3 months to 40 subjects with knee OA. The effect of HA was measured using a visual analogue scale (VAS), the WOMAC total score and the WOMAC pain score, and by observing inflammatory cytokine, bradykinin and leptin levels. The authors showed that oral consumption of HA significantly reduced pain and decreased cytokine, bradykinin and leptin release [45].

A study comparing intra-articular injections and oral administration of HA was recently published [46]. Two groups of subjects with early OA were administered three weekly intra-articular injections of HA or tablets containing 300 mg of HA plus *Boswellia serrata* extract 100 mg (first 20 days) or 150 mg of HA (remaining 20 days). Patients were evaluated using the American Knee Society Score (AKSS) and VAS and were correlated to subject age. Both groups showed beneficial effects, with intra-articular injections found to be more effective for those under 60, while oral administration was better for patients above 60 [46].

N-acetyl-D-glucosamine

NAG is a glucose derivative. It is an amino water-soluble monosaccharide, resulting from the linkage of glucosamine (GA) to acetic acid, and polymerizes linearly with (1,4)-β linkages. NAG is abundantly present in the body and is the natural precursor of HA. It is also a component of cartilage matrix and synovial fluid. Because of its physiological role, GA derivatives are used as nutraceutical supplements for chondroprotection.

Mechanisms of action are still under investigation. It has been demonstrated on synovium explants that glucosamine hydrochloride (GH) increases HA production, but that NAG does not [47]. A similar result was found by Igarashi et al who reported that NAG does not promote HA synthesis in synovial cells and chondrocytes [48]. However, NAG upregulates the hyaluronan synthase-2 in human articular chondrocytes [46] and mediates anti-inflammatory pathways [49, 50], reducing nitric oxide, cyclooxygenase-2 and IL-6 pro-

duction [49]. Shikhman et al suggested that NAG could be more efficient than native GA in HA synthesis [51].

GA has a lower molecular weight than HA and so has better absorption and bioavailability. Since GA is naturally present in the human body, it is difficult to conduct pharmacokinetics studies. However, in vitro and animal studies demonstrate intestinal internalization of GA [52–54]. In 1972, Tesoriere et al suggested that NAG is absorbed through a diffusion process [55]. In rats it has been demonstrated that radiolabelled NAG shows a peak of adsorption 4 hours after administration and that residual activity is maintained for up to 168 hours [54]. The bioavailability of GH in dogs is about 10–12% [52]. Persiani et al [56] corroborated this finding in 12 humans who each day received glucosamine sulphate (GS), which was quickly absorbed after oral administration, showing a linear pharmacokinetics profile with doses ranging from 750 to 1500 mg and an estimated half-life of 15 hours. Most pharmacokinetic investigations are concerned with GS.

Clinical evidence for glucosamine

GA has been included in medical preparations for nearly 40 years [57] and GA derivatives are commonly used to treat OA. However, the indications for its use, mechanism of action and effects on OA have not yet been fully defined, although over the past decades several meta-analysis have been carried out to assess the effectiveness of GA.

In 2000, McAlindon et al [58] conducted a systematic quality assessment and meta-analysis of trials found on MEDLINE and in the Cochrane Central Register of Controlled Trials, to investigate the conflicting outcomes following the use of GA in patients with OA. Their findings suggested that GA helps relieve OA-associated pain, but that the effects are exaggerated [58].

In 2003, a comprehensive meta-analysis by Richy et al assessed the effectiveness of GA. Evaluation of 15 studies concluded that GA exerted positive outcomes on all tested parameters, such as joint space narrowing, the Lequesne index and WOMAC score [59].

A meta-analysis of GA use to treat OA was published in 2005 on the Cochrane Database of Systematic Reviews. The authors concluded that there were no significant differences between non-Rotta preparations and placebo, that Rotta preparations improved pain and muscle functionality, and that there no differences in stiffness resolution between the use of a GA-containing preparation and placebo [60].

More recently, Wandel et al showed that GA, chondroitin, or the two in combination did not significantly reduce pain

and joint space narrowing. Their network meta-analysis was conducted on 10 studies [61].

The American College of Rheumatology, American Academy of Orthopaedic Surgeons and Osteoarthritis Research Society International recommend that GA, chondroitin or a combination of both should not be used for pain associated with OA. However, in 2015, Zeng et al [62] investigated the impact of GA plus chondroitin, GA alone and celecoxib on relief of knee OA. The analysis covered 54 studies and concluded that both GA plus chondroitin and GA alone have beneficial effects on pain and result in functional improvement [62].

In 2006, Hatano et al showed that a soymilk drink containing 1,000 mg or more of NAG and consumed once daily was beneficial for patients with knee joint impairment. After 2-month treatment, pain was reduced and range of motion was improved [63].

Tsuji et al showed in 2016 that NAG was effective for improving knee function. A randomized, double-blind, placebo-controlled trial was conducted on 50 patients aged 52–87 years with knee pain. Subjects were administered 100 mg of NAG and 180 mg of chondroitin sulphate daily for 6 months and the effects were evaluated using the JKOM [64]. Beneficial effects on functional knee activity were first noted at the 3-month evaluation [64].

Several randomized, double-blind, placebo-controlled studies have recently been conducted by the same research group on healthy volunteers to assess the role of NAG and its effective dosage. The trials investigated 16-week supplementation in subjects aged 20–64 years and concluded that 500–1,000 mg/day of NAG improved type II cartilage metabolism, promoting cartilage synthesis and reducing cartilage degradation [65–67].

Naraoka et al evaluated the impact of NAG administration in subjects with knee discomfort but without a diagnosis of knee OA. Nineteen adults were given a tablet containing 526.5 mg of NAG and 33.6 mg of proteoglycan (PG) thrice daily for 3 months. Locomotion was improved and pain was reduced after treatment. The authors suggest that early administration of NAG and PG could prevent structural knee deformation, and thus the onset of OA [68].

Discussion and conclusion

OA is a common degenerative disorder that dramatically affects the quality of life of patients. It damages the entire joint, triggering cartilage and juxta-articular tissue changes and biomechanical stress, which lead to the loss of articular cartilage, histological abnormalities in the synovia and joint capsule, and muscle weakness. These changes limit mobility, interfere with daily activities, cause pain and reduce the overall quality of life.

HA and NAG can significantly reduce OA progression. Over the last two decades, several studies have examined their efficacy after oral administration in reducing the pain and stiffness associated with OA, but the overall results are controversial.

Oral HA supplementation exerts beneficial effects on OA discomfort. Daily intake of doses ranging from 40 to 300 mg of HA alleviated pain [39, 45] and improved physical function [38, 41]. HA supplements and yoghurt enriched with HA had positive effects on pain and muscle strength. Conversely, oral administration of the HA natural precursor NAG does not exhibit the same positive results. The ability of GA to reduce pain and structural changes in OA has been widely investigated. However, most studies do not specifically consider the effectiveness of oral administration of NAG, even though it is the natural precursor of HA.

Most trials are on GS or GH, although NAG is widely used in food supplements to relieve joint discomfort and counteract functional knee changes. Meta-analyses on GA have not reached definite conclusions. McAlindon et al suggest it has beneficial effects, but claim results are exaggerated [58], Richy et al and Zeng et al found health improvements [59, 62], but Wandel et al did not [61], while major guidelines even discourage the use of GA in OA [62]. Conversely, the few papers related specifically to NAG find it improves knee function [64–68], although its mechanisms of action are still under investigation, and in some cases, results concerning its role in improving HA synthesis are conflicting [46–48, 51]. In Italy, the role of NAG in HA synthesis is confirmed by the Ministry of Health, which in food supplements containing NAG allows the claim that it contributes to HA synthesis. Summarizing, the role of NAG in non-pharmacological OA management needs to be better elucidated in comparison with HA outcomes.

To conclude, these observations concerning bioavailability are unexpected. Bioavailability and efficacy are closely connected since an active compound must survive the gastrointestinal tract and reach the circulation unmodified to exhibit a therapeutic effect. NAG is a small monosaccharide and few pharmacokinetics studies [54, 55] have investigated its absorption since it is widely present in the body and has a low molecular weight. Indeed, its enteric absorption is taken for granted. On the other hand, HA bioavailability is still the subject of investigation, even though its beneficial

effects have been confirmed by the scientific literature. As the high molecular weight of HA limits intestinal absorption, the mechanism for the positive clinical outcomes has still to be elucidated. Our current knowledge suggests oral administration of NAG should show better effects than that of HA. However, Kimura *et al* speculated that prior to absorption, HA is cleaved into oligomers by bacteria in the lower intestine [33]. To conclude, further investigations are need to clearly elucidate the role of HA and NAG in OA non-pharmacological treatment, and whether HA derivative oligomers could explain these controversial outcomes.

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