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Review article

Hyaluronic acid and articular cartilage

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Abstract

Osteoarthritis is a typical locomotive-syndrome-induced disease. Osteoarthritis (hereinafter referred as "OA") is defined as a progressive disease characterized by cartilage degeneration; due to the changes in regeneration and proliferation of articular cartilage and bone, and secondary synovitis induced by degeneration of articular cartilage and physical abrasion. It has been clarified that aging, physical stress and oxidative stress induce the composition changes of cartilage matrix substrate and consequently, disorders of cartilage matrix are induced. This review outlines mechanism how disorders of cartilage matrix are induced in an early stage of OA onset, focusing on hyaluronic acid (hereinafter referred as "HA"), which is a component of matrix. It has been reported that based on the early stages of OA patients and OA model animals, HA becomes fragmented, cartilaginous tissues migrate, and matrix-degrading enzymes are activated, which is normally restricted by HA. As the result, disorders arise in articular cartilage substrate matrix. Administration of exogenous hyaluronic acid (HA) can mitigate these reactions to some extent. The damaged articular cartilage releases proinflammatory cytokine and matrix-degrading enzymes such as HA degrading enzyme and aggrecan degrading enzyme are induced. It appears that exogenous hyaluronic acid has a function of inhibitory against induction of matrix-degrading enzyme and mitigate migration of matrix from cartilage (loss of HA and aggrecan). However, American Academy of Orthopaedic Surgeons (AAOS) announced a policy that intra-articular administration of HA is not recommended. For prevention of OA, it is essential to clarify the disorder mechanisms of cartilage matrix, identify initial symptoms in as early stage as possible. Therefore, countermeasures are enabled to be conducted to prevent of onset and progression of OA in the early stage.

***KEY WORDS*:** hyaluronic acid, hyaluronan, cartilage degeneration , osteoarthritis, aggrecan

Introduction

Our research center has participated in SIP (Cross-Ministerial Strategic Innovation Promotion Program). SIP promotes industry-academia collaboration to develop functional agricultural, forest and fishery products and food products for the next generation, which are effective to maintain function for physical locomotion. With this goal, multiple major food companies in Japan provide potential or promising food ingredients, participate with their technology and industry-academia research teams has been established.

The background of this project is that the elderly in this rapidly increasing aging society, Japan, have compelling needs to maintain and improve the quality of life (QOL), which could result in the energy source of whole the society. These days the elderly persons who have certifications of needed long-term-care have rapidly increased in number, who have difficulties to live a self-supported life, as their functions of daily life motion (physical locomotion) are lowered. Thus, it is extremely urgent to tackle for countermeasures for prevention.

A risk of locomotion syndrome is osteoarthritis (OA). It enables to prevent of the onset and progression of OA that signs of anomaly in articular cartilage substrate matrix should be found at an early point. Furthermore, Hyaluronic acid (HA) and glucosamine are effective as functional food, which have been produced on a commercial basis focusing OA-induced knee pain, arthralgia and lower back pain, lumbago. It has been reported recently that glycative stress is involved in the mechanism of rheumatoid arthritis

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(hereinafter referred as "RA")[1-5] and OA occurrence mechanism. Further, it is suggested that HA is related to the mechanism. This paper outlines the knowledge and information related to HA and OA. The tendency of the term of HA in Europe and the United States is to designate not hyaluronic acid but hyaluronan, which means scaffold. In this paper, both of the terms are described as HA.

Aggrecan in articulation

Articular cartilage structure is shown in ***Fig. 1***. Aggrecan, which is a member of proteoglycan family, is a main component of cartilage, filling intercellular space. Aggrecan is a compound material of glucose and protein with a molecular weight of approximately several hundred thousand Dalton and has a diversified functions as a biogenic substances. Producing matrix with collagen and HA (***Fig. 2***), aggrecan maintains cartilage and other articulation tissues (***Fig. 3***). These components are functional components to maintain and repair tissues, playing roles of system constitution and transmission material. The polysaccharide moiety is called glycosaminoglycan, which is composed of chondroitin sulfate, heparan sulfate, keratan sulfate and dermatan sulfate[6-8].

As for distributions, HA are mainly is distributed in surface cell space and also present in connective tissue of a hollow space surrounding blood vessels. Chondroitin sulfate is distributed in highly fibrotic parts and connective tissues surrounding blood vessels. Dermatan sulfate is distributed in surface interstitial tissues and blood vessel endothelial cell and heparan sulfate is distributed in blood vessel endothelial cell[7].

Articular inflammation was induced by rheumatoid arthritis (RA) and osteoarthritis (OA) and also aging-caused regressive changes occur. Consequently, aggrecan and HA are localized and change qualitatively and quantitatively. Aggrecan comprises two groups; one contains chondroitin sulfate affluently and the other contains keratin sulfate affluently. The latter increases along with aging and changes the association process state with HA[9].

It is evident that senescence-accelerated mouse (SAMP8 and SAMR1) researches shows decreased dyeability of type I collagen and HA-binding protein (HABP) and increased dyeability of type II and type X collagen in the temporomandibular articulationcaput mandibulae cartilage[10], which are induced by aging.

Degradation of agrin matrix is related to the process in articular cartilage destruction of OA. Articular extracellular matrix constructs a higher order structure by the interaction of HA-aggrecan network and type II collagen fiber[11]. HA binds to aggrecan to protect articular cartilage from load and to exert lubrication effect on the surface of cartilage[12]. In the articular cartilage destruction process, HA-aggrecan network is degraded and then collagen fiber degradation is proceeded. Extracellular matrix is degraded with MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) of a gene family playing a significant role[11]. In an early stage of the articular cartilage destruction, ADAMTS4 and ADAMTS5, which are called aggrecanase, play a leading part of aggrecan degradation and HYBID (hyaluronan-binding protein involved in hyaluronan depolymerization: KIAA1199) plays an important role of HA degradation.

In this manner, metabolism of cartilage and bone is supported by the balance of generation and degradation of matrix. Mechanical stress, oxidative stress and inflammation induce degrading enzymes, which causes high turnover metabolic bone deterioration.

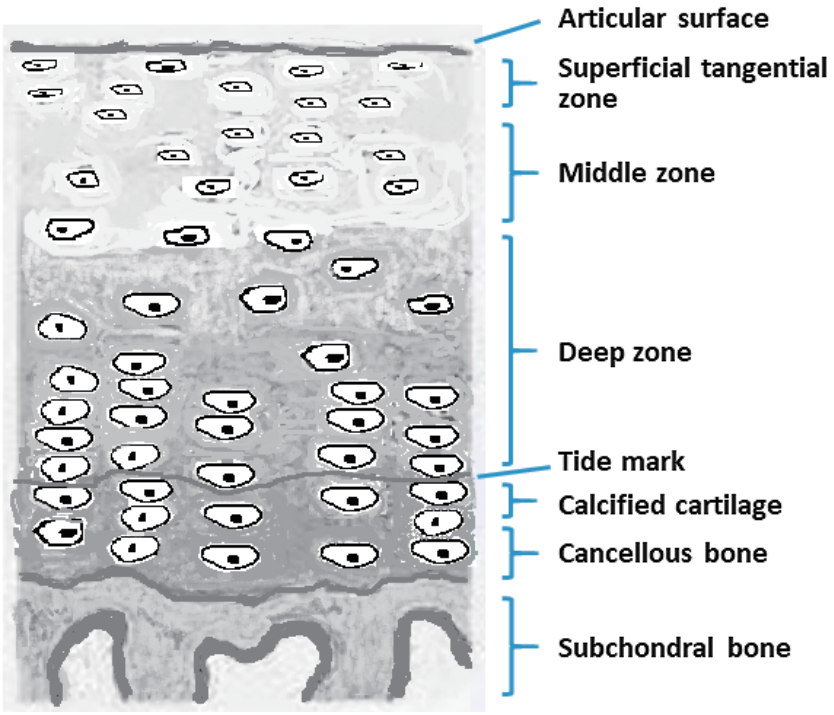

Fig. 1. **Schema of cartilage tissues.**

Cartilage tissues areresilientfibrous connective tissues and consist of cartilage cells, cartilage matrix of intercellular substances and perichondrium covering cartilage.

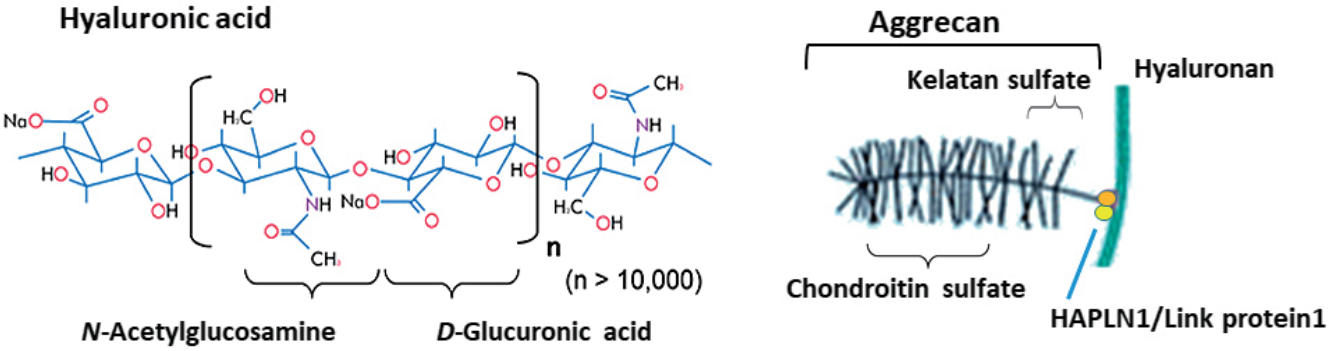

Fig. 2. **Structure of hyaluronic acid.**

HA has thestructure of bound disaccharide unit of N-acetylglucosamine and D-glucuronic acid, with a high molecular weight, of more than several hundred thousand, often reaching millions. HA, hyaluronic acid.

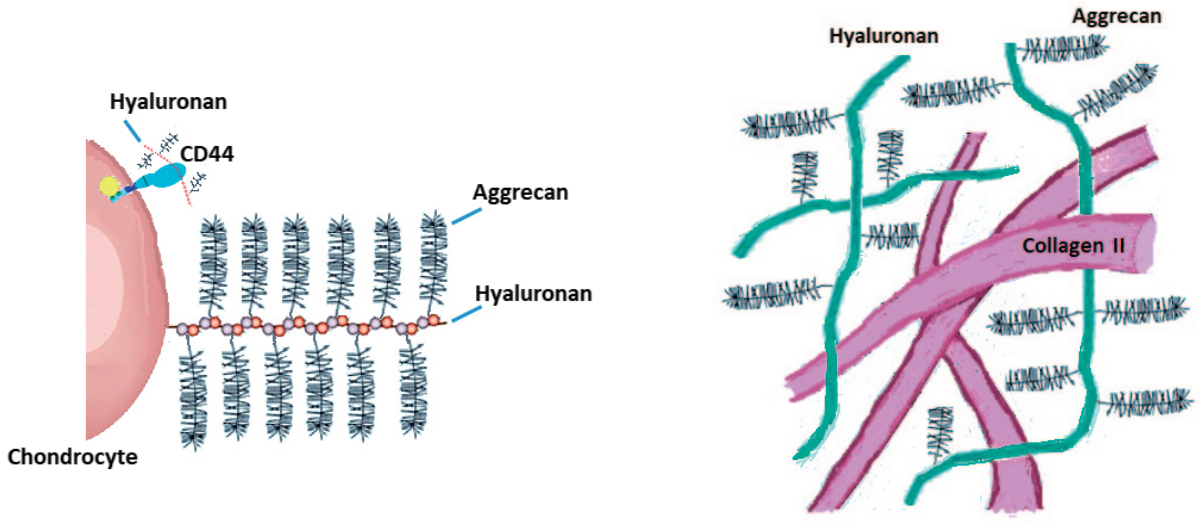

Fig. 3. **Configuration of articular cartilage matrix..**

Aggrecan is a cartilage-specific proteoglycan core protein or chondroitin sulfate proteoglycan/keratan sulfate proteoglycan with a molecular weight of 2,500 kDa. Aggrecan is formed in a manner where core protein binds with keratan sulfate/ chondroitin sulfate. Aggrecan bindswith hyaluronic acid through HAPLN1/Link protein1.Hyaluronic acid functions as scaffoldingfor thematrix and binds to cartilage tissues through CD44. Hyaluronic acid, also through integrin and ICAM-1, binds to cartilage tissues. Normal hyaline cartilage consists of type II collagen microfibril and aggrecan. HAPLN1, hyaluronan and proteoglycan link protein 1; ICAM-1, intercellular adhesion molecule-1.

Hyaluronic acid

HA is widely distributed throughout the body tissues and organs. HA is produced from articular synovia in articulation and is a main component of synovial fluid. Other than that, HA is the component of articular cartilage aggrcan [13]. Water retentively [14-16], lubricant of articulation [12, 17-21], intercellular adhesion [22,23] and immune regulatory activity [24] are significant roles for HA to play.

HA exists in human blood and is clinically used as an index for osteoarthritis (OA) and rheumatoid arthritis (RA) and also an index of hepatic fibrogenesis. Blood concentration of HAindicates as follows;

Healthy persons; 30.1 ± 16 ng/mL, RA patients; 220 ± 204 ng/mL, OA patients; 55.3 ± 31 ng/mL [25]

Healthy persons; 42.2 ± 45.6 ng/mL, RA patients; 372.5 ± 401.2 ng/mL [26]

Healthy persons; 33.7 ± 24.2 ng/mL, RA patients; 350.7 ± 689.5 ng/mL [27]

RA patients showed higher values. Blood concentration of HA has correlation with CRP, WBC and progression stage. HA serum concentration increasesin OA along with pain symptoms and progression of diseases and increases in RA along with advancement of articulation destruction [13].

HA concentration of synovial fluid of healthy persons (at the average age of 27.5) is 3.4 ng/mL [28]. HA concentration of synovial fluid decreases along with aging in healthy persons. However, HA concentration of synovial fluid of OA patients does not show changes due to aging [29-31].

OA patients tend to have lower HA concentration of

synovial fluid than healthy persons. When the amount of synovial fluid increases, HA concentration decreases [32, 33]. The reason is assumed that in OA, cartilage destruction lowers HA production.

HA concentration of synovial fluid sometimes indicates lower in RA than in OA [34]. HA concentration of synovial tissue extracted solution is higher in RA than in OA, which is induced by the HA production acceleration of synovial tissues proliferated due to RA [35, 36]. In the early and active stage cases of RA patients' synovial cells, the production of hyaluronidase, which is an enzyme to cleave HA, is elevated. In cases where hyaluronidase activities are high, HA is degraded to be lowered in quantity and quality, which causes low-viscosity synovial fluid [37].

Cell culture Experiments

Researches which has been reported are expounded in three categories, cell culture experiments, animal experiments and clinical trials. Cartilage matrix degradation product is called matrikine, which induces matrix-degrading enzyme to destruct cartilage. As a result, articular disorders are induced. The macromolecular hyaluronic acid infiltrates damaged cartilage of OA and RA and binds with HA receptor on the surface of cartilage cells and inhibits cytokine and matrikines from catabolism. HA has a pharmacological effect to prevent cartilage destruction [38].

Experiments have been conducted to stimulate cultured cartilage cells and to reproduce OA. Cartilage cells shows varied cell responses in being stimulated by oxidative stress (*i.e.*oxygen-derived free radical [ODFR], inflammation, inflammatory cytokine (IL-1α, IL-1β, TNFα), collagen fragment derived from cell destruction and RA synovial fluid. The stimulus activates p38MAP kinase, activates nuclear factor-kappa B (NF-κB), promotes phosphorylation and nuclear translocation, produces MMP (MMP-1, MMP-3, and MMP-13) and increases secretion extracellular [39-41]. A kind of C-C chemokine, RANTES [42], ADAMTS4 [43], which has degradation activity of aggrecan and prostromelysin [44], are materials which are produced by stimulus of IL-1α. The production of TIMP-1, which is a tissue inhibitor and has a function to protect cartilage, is inhibited [44].

IL-1β promotes the destruction of cartilage matrix. Furthermore, IL-1β upregulates the expression of RHAMM mRNA of cartilage tissues [45], and decreases the expression of α2(VI), α1(II), α1(IX) and α2(XI) collagen genes, which involve in the production of collagen [46,47]. IL-1β affects cartilage tissues and increases the expression of CD44 [48, 49].

In cartilage tissue, inductive NO synthetic enzyme (iNOS) is induced by the stimuli of fibronectin fragment, which contains binding-site of C-terminal heparin and the production of NO increases [50]. Changes in concentration of intracellular calcium ([Ca2+]i) involves theses reactions [51]. HA acts inhibitively these reactions.

In normal cartilage tissues, HA has a high affinity with cartilage surfaces and cannot be absorbed; HA can be absorbed into the deep zone of OA cartilage [52].

HA has very little effect on the synthesis of aggrecan in cartilage tissues [53, 54], but inhibits the migration of aggrecan from cartilage tissues [52, 54, 55]. Rooster comb-derived HA is also recognized to inhibit the migration of aggrecan [54].

HA inhibits the migration of aggrecan and the production of MMP through CD44, which is a main receptor on the surface of cartilage tissues [39]. In cartilage tissues, cell adhesion molecules (ICAM-1) are regarded as HA receptors. HA inhibitory effects against the production of MMP, which is collagen-fragment induced, are exerted through ICAM-1 [56]. HA promotes the production of the tissue inhibitors metalloproteinase-1 and TIMP-1 in cartilage tissues [57]. HA increases stromelysin activity but decreases the fraction of stromelysin/TIMP-1 [57]. HA inhibits the fibronectin segregation from cartilage tissues [58].

It has been reported that exogenous hyaluronic acid inhibits the production of ADAMTS4 due to cartilage tissue IL-1α stimuli [43], and inhibits the decline of the gene expression of collagen alpha-2(VI) due to IL-1α stimuli [46, 47].

Comparing normal cartilage and OA cartilage, in OA, the production of NO by HBFN-f is exacerbated and CD44 is upregulated [59]. HA inhibits the acceleration of NO production through the coaction with CD44.

The expression of RHAMM mRNA is upregulated by IL-1β and TNF-α in cartilage tissues [60]. In an examination of HA receptors (CD44) and receptors for hyaluronan-mediated motility (RHAMM) in knee synovial membrane tissues of OA patients, the results showed that progressive OA patients had more strongly dyed cells than healthy subjects. Furthermore, expressions of both CD44 and RHAMM were stronger in OA patients than healthy subjects, using Western Blotting Methods [61]. This finding revealed that the onset and progression of OA involved the changes in the level of hyaluronan-binding protein.

HA also affects the differentiation and maturity of cartilage cells [62]. An appropriate amount of HA promotes the differentiation of cartilage, but adding high concentration of HA in other conditions inhibits the differentiation of cartilage. In the differentiation processfrom cartilage stem cell to cartilage cell, gene expressions related to chondrocyte differentiation, aggrecan and Sox9, are elevated, and gene expressions of CD44, TGF-β1 and hyaluronic acid synthetic enzyme 2 are elevated. The quantity of HA and GAG in culture supernatant are increased [63]. It is evident that exogenous hyaluronic acid promotes the production of HA by the mechanism of autocrine and paracrine and also promotably affects chondrocyte differentiation.

Depolymerization of hyaluronic acid by a hyaluronidase treatment accompanies the increase in MMP expression and upregulation of CD44, and induces the destruction of cartilage [64]. HA depolymerizationis induced by hydroxyl radical, which is reactive oxygen species [65]. It is assumed that in the case where conditions of strong oxidative stress are continuously present, or in the case of hyaluronidase activity acceleration, exogenous hyaluronic acid fragments could induce adverse events.

HA is produced mainly by synovial membrane cells. In OA and RA, cytokine in synovial membrane cells increases [38]. Periostin, which isone of the synovial-membrane-cell-derived cytokines, is a key molecule of inflammation and cartilage degeneration in OA. Periostin promotes the acceleration of NO generation to cells of articular tissue and elevates the expression of inflammatory cytokine and MMP. Periostin-dependent NO generation is inhibited concentration-dependently by HA [66]. IL-13 stimuli increases the productivity of periostin in synovial membrane cells, and HA has an inhibitory effect on this [67].

Animal experiment

It has been reported that in animal experiments, intra-articular administration of macromolecule HA has an effect of pain relief [68-75]. It is assumed that molecular conformation characterized by macromolecule HA greatly contributes to it. Mechanisms of pain relief are assumed as follows [68];

1) HA coats pain receptors of tissues, such as the synovial membrane.
2) Endogenous pain producing substance is captured.

In articulation of a cow model for OA with pressure stress, the following are shown; ROS generation increases, gene expressions of type II collagen and aggrecan are inhibited [76], inhibitory HA exerts an anti-oxidative effect and inhibits the generation of reactive oxygen species (ROS) [77], matrix synthesis control element (SOX9) is decreased in the expression by pressure load, and adding HA restores it [76]. Also, phosphorylated P38 and MMP-13 increase in expression by pressure load, and adding HA inhibits it [76]. Mechanical stress to cartilage cells induces ROS synthesis acceleration and P38 MAPK phosphorylation. Finally, articular cartilage degeneration is induced through the inhibition of matrix synthesis and the promotion of MMP13 generation. Contrarily, it is assumed that HA has a mechanism to exert effects of protection of cartilage by the inhibition of ROS production and P38 MAPK phosphorylation through the receptor, CD44 [76].

It has been reported that in experiments with a Rabbit model of OA, the expressions of VEGF and VEGFR-2 mRNA increase. However, HA intra-articular administration has small effects on the expression of VEGF-2 mRNA, while inhibiting the expression of VEGFR-2 mRNA [78]. HA administration inhibits the generation of PGE_2 and MMP [79]. Proteoglycan, type II collagen and a residual quantity of HA result in a decrease, and a positive rate of apoptotic cell and degradation product of aggrecan results in an increase. However, macromolecule HA administration to a joint cavity corrects these changes [80].

Iodoacetic-acid-induced OA rats were examined to analyze HA response genes of DNA microarray in cartilage tissues. Results showed that the gene expression of type IV, IX, XI collagen and adrenomedullin decreased due to OA and macromolecule HA administration intra-articular administration restored them. Inflammation-related factors such as phospholipase A2 and Toll-like receptor 8 were accelerated in gene expression due to OA, and macromolecule HA administration inhibited those [81].

It was suggested that macromolecule HA intra-articular administration affects clinical conditions to control the gene expression of collagen, anti-inflammation factors and inflammation-related factors [81].

It is evident that HA action mechanism of the inhibition of OA progression is partly related to the inhibition of VEGF mRNA expression [79].

In spite of the results of these researches, the American Academy of Orthopaedic Surgeons, AAOS, released a revised guideline of knee osteoarthritis in June of 2013 and announced that intra-articular HA administration was no longer recommended as a method of treatment for patients with symptomatic osteoarthritis of the knee (recommendation grade: strong). Also, the Osteoarthritis Research Society International (OARSI) has a negative advocacy piece against intra-articular HA administration.

Clinical study

Some clinical studies have revealed the pain relief effects of HA oral ingestion. Processed food from rooster comb extracts containing low molecular HA was examined in a randomized double blind comparative trial; including low molecular HA, and HA degraded to a low molecular weight. Subjects were 40 patients with knee osteoarthritis (OA). In Japanese Orthopaedic Association (JOA) Evaluation Criteria, 2 of 5 subscales and total scores were significantly improved for “pain and ability on walking” and “pain and ability on ascending and descending stairs” [82, 83]. Furthermore, in a randomized double blind comparative trial examining rooster comb extracted processed food containing low molecular HA with 66 soccer athletes, a tendency toward improvement was shown in “pain when placing pressure on foot joint,” and “pain of articulation coxae while exercising” [84]. An examination of prepared soybean milk mixed with N-acetylglucosamine was conducted in a randomized double blind comparative trial, where 67 subjects with mild pain, stiffness and discomfort pain in the knee joint showed that “pain in the knee joint when ascending and descending stairs and during resting period” were significantly improved [85]. In a clinical study onthe ingestion of glucosamine-chondroitin-quercetinglucoside with 46 OA patients and 22 RA patients, OA patients showed a reduction of pain but RA patients did not show a reduction of pain [86].

These results of clinical studies have indicated the possibilities that HA with a relatively small molecular weight are effective on OA-derived arthralgia. There are a certain amount of unsolved questions remaining regarding action mechanisms for how low molecular HA is digested, degraded and absorbed after oral ingestion, and also how to mitigate pain.

As for influences on the range of joint motion by HA, research of OA rabbit models with knee arthrodesis has been reported. Macromolecule HA administration into a joint cavity mitigates the lowered range of knee joint motion [87]. HA with a molecular weight of 2.02 million was more effective than HA with a molecular weight of 0.95 million. There has been no research reported regarding a low molecular HA like this specimen. The HA mechanism has not yet been completely clarified. However, some mechanisms have been assumed such as, HA has an inhibitory effect against fibrosis, involves water retaining action [88], elevates the fluidity and restores the function of synovial fluid [89], and at the same time, inhibits the separation of glycosaminoglycan, which is a component of cartilage tissue and restores the quantity of glycosaminoglycan in the cartilage, suppressing the progress of cartilage degeneration [90-92].

In articular cartilage tissues stimulated by adding IL-1 and RA, aggrecan is promoted to free to extracellular and the synthesis and secretion of MMP is induced. These reactions are forced by HA [39]. It has been confirmed in research of articular cartilage cultured cells of rabbit models that HA had little effect on the synthesis of aggrecan but inhibited aggrecan to separate from the substrate [53].

A clinical study of a soymilk beverage containing N-acetyl glucosamine on OA in a double blind parallel comparative trial revealed that the range of joint motion was significantly improved after 8 weeks from the ingestion [85].

Relationshipbetween glycative stress and HA

The relationship between HA and glycative stress have not been paid a full attention to so far. However, it has become known that glycative stress involves the mechanism of pathogenesis of OA [93-96]. Mechanisms are assumed to be as follows; DAMPs (damage-associated molecular patterns) and HMGB-1, which are released from impaired cells into a joint cavity, promote the generation of inflammatory cytokine, through the mediums of TLRs (toll-like receptors) and RAGE (receptor for advanced glycation end products) on immunocompetent cells. The induced inflammatory tissue injury leads to the onset and progression of OA [96]. In an OA joint cavity, AGEs (advanced glycation end products) induce inflammation through the mediums of RAGE and other scavenger receptors [95]. An examination of serum malondialdehyde concentration of a rabbit OA model showed as follows; control group (untreated); 2.05 ± 0.37 nmol/mL, hyaluronan group; 1.94 ± 0.54 nmol/mL, cortisone group; 1.98 ± 0.37 nmol/mL and hyaluronan and cortisone combination group; 1.55 ± 0.41 nmol/mL. The combination group was significantly lower than the untreated group [97]. Malondialdehyde acts as an intermediate in the process of the formation of AGEs and has an effect of accelerating the formation of AGEs. Therefore, this view is striking in terms of relating HA to glycative stress.

An experiment of administering feed containing HA to rats revealed that the ingestion of HA exerted thebeneficial influences to intestinal bacterium flora and serum cholesterol metabolism [98]. The data proved a significant decrease of TC in this test. The relation between HA and glycolipid metabolism is of great interest, and further research is expected for the future development.

Conclusion

The cartilage matrix plays an essential role to consider OA pathophysiology. Focusing on HA, which is a matrix component, it is concluded that mechanical stress, oxidative stress and glycative stress induce HA fragmentation and the migration of cartilage tissues in early stages of OA, and the decline of HA induces the activation of a matrix degrading enzyme, which is normally suppressed by HA. These processes are factors of a matrix disorder. Exogenous HA can act suppressively on the enzyme induction, and as a result, reduces the matrix separation (the loss of HA and aggrecan) from cartilage. However, differences in effects, depending on the molecular size, have not been clarified. It is necessary for the prevention of OA to elucidate the mechanisms of cartilage matrix disorders and to enforce countermeasures for the prevention of OA progression, identifying indications of early symptoms.

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Conflict of interest statement

There are no items deemed to be conflicts of interest in this research.

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