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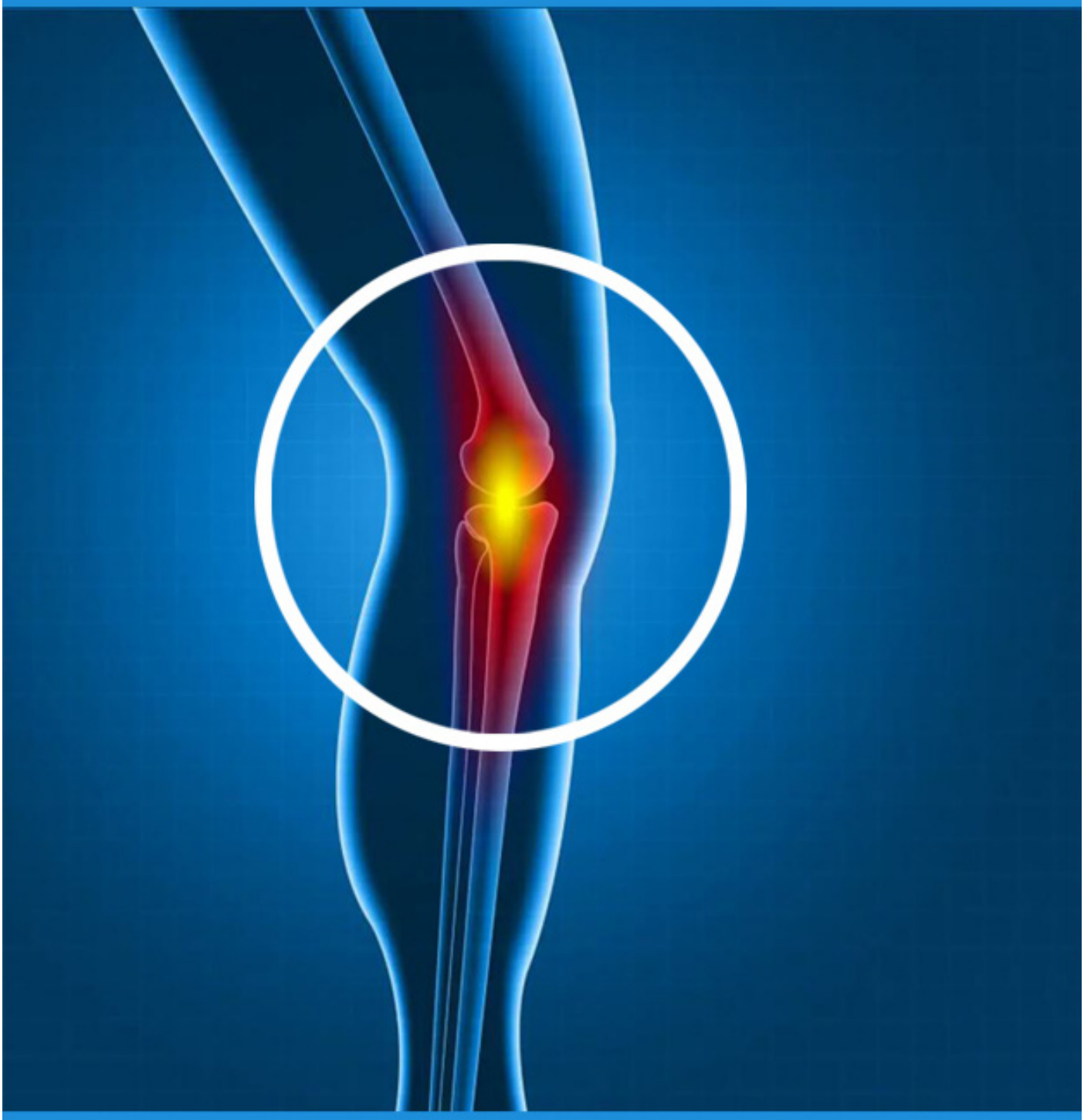

Trehalose: A promising Stabilizer Agent for Hyaluronic Acid

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Abstract

Hyaluronic acid has long been used as an infiltrative therapy for osteoarthritis. Besides its physical properties of shock absorption, one of its primary effects is to reverse the inhibition of proteoglycan synthesis and attenuate osteoarthritis's oxidative stress reaction cascade. However, clinical trials provide controversial results regarding its effectiveness. One possible explanation is its fast clearance rate due to reactive oxygen species. For this effect, different kinds of molecules have been proposed as hyaluronic acid stabilizer agents, with promising results not only for delaying its clearance but also as enhancers of the anti-inflammatory effects. It has been demonstrated that trehalose is one of the best alternatives for stabilizing hyaluronic acid by neutralizing hyaluronidase. Furthermore, it has shown promising in vivo results by effectively achieving longer-lasting effects than a non-trehalose hyaluronan. This chapter reviews the main metabolic events in osteoarthritis disease and trehalose as a insights as a valid alternative for optimizing treatment results.

Introduction

Osteoarthritis (OA) is one of the most prevalent degenerative joint diseases; Characterized primarily by joint pain and functional impairment, produced by articular cartilage degeneration, subchondral bone remodeling, and synovial inflammation [1]. According to the 2019 Global Burden of Disease Collaborative Network study, osteoarthritis affects 7% of the global population and is the 15th highest cause of years lived with disability worldwide [2]. In addition, the constant aging population, increasing obesity, and the presence of metabolic disease intensify this phenomenon.

OA syndrome is multifactorial, complex, and patient-dependent. Often, there will not be one single cause but will depend on personal factors like sex, genetic inheritance [3], physical activity; (i.e., high-impact sports) [4,5], and mechanical factors like knee mal-alignment or previous knee injury. In addition, OA has increasingly been correlated with patients' metabolic conditions, including visceral obesity, insulin resistance, low-HDL cholesterol, and high triglycerides [6]. Interestingly, a strong correlation between atherosclerosis and cardiac infarct has been made; all of them are part of metabolic syndrome, which has recently been known as an independent risk factor for OA [7,8].

With increasing knowledge of OA pathogeny and its metabolic cascade, treatment strategies have focused on disease prevention and early treatment rather than end-stage disease solutions. Hyaluronic Acid (HA) has been proposed as a promising infiltrative therapy for the osteoarthritis inflammatory

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process slowing its progression. However, because of the controversial results of clinical trials, constant advances are being made to optimize HA effectiveness. Rapid clearance due to Reactive Oxygen Species (ROS) could be the cause that limits its effectiveness. For this reason, different kinds of polyols and molecules have been proposed as hyaluronic acid stabilizer agents, with promising results for clearance delay and enhancers of anti-inflammatory effects. This chapter will discuss OA physiopathology, emphasizing its metabolic pathogeny, hyaluronic acid mechanism of action, and the novel trehalose-enhanced hyaluronic acid, which is thought to maximize effects through neutralizing hyaluronidase enzyme activity as well as decreasing reactive oxygen species.

Osteoarthritis metabolic pathogenesis

Osteoarthritis is a joint disease involving structural damage in the hyaline cartilage, subchondral bone, ligaments, capsule, synovium, and periarticular muscles. The complex pathogenesis of osteoarthritis involves mechanical and inflammatory metabolic factors, which ultimately lead to structural destruction and failure of the joint. This disease is characterized by an active dynamic state produced by an imbalance between repair and destruction of articular tissues.

The joint damage produced by the released inflammatory cytokines after an external trauma (i.e., IL-1β, TNF-α), as well as a persistent inflammatory metabolical syndrome, or the cellular senescence caused by aging lead to the same pathway of articular degeneration; In which one of the main events is mitochondrial dysfunction, resulting in increased ROS production and the triggering of an apoptosis cascade [9]. The final result is a persistent oxidative stress environment. On the other hand, antioxidant enzymes (such as SOD, CAT, GPX, and PON1) are decreased in OA patients, confirming the role of oxidative stress in OA pathogenesis [10,11].

Autophagy's pathogenic mechanism of joint destruction has become of increased interest in the last few years. This term refers to the intracellular mechanism through which cells remove defective organelles and macromolecules to protect themselves against cellular stresses. With senescence, the autophagic activity of cells decreases; subsequently, there is an alteration of the clearance efficiency, with aggregation of macromolecular proteins, leading to cellular dysfunction, degeneration, and apoptosis [12]. Not only does senescence cause alteration in autophagy, Cetrullo et al. found that autophagic flux was also inhibited under oxidative stress in human chondrocytes [13], on the other hand, autophagy stimulation protected chondrocytes from degradation [14] (Figure 1). Furthermore, the trehalose disaccharide molecule has been shown to produce an upregulation in the autophagy protective mechanism [12], which will be further discussed.

Inside the joint, this cyclic oxidative stress produces destructive effects on tissues altering their functioning. For example, degradation of extracellular matrix (ECM) occurs along with a decrease in its ability of chondrocyte synthesis. On the other hand, chondrocytes undergo senescence and apoptosis. In addition, intraarticular hyaluronic acid is directly converted into oligosaccharides, thus reducing its viscosity and molecular weight. Oxidative stress also causes changes in the function of osteoblasts and osteoclasts in subchondral bone, which furtherly worsen OA progression.

By this means, persistent oxidative stress should be the target treatment in OA, simultaneously with the correction of the triggering cause; this will decrease pain and inflammatory response while stopping from suffering further damage. This effect could be potentially reached with trehalose enhanced hyaluronic acid.

Figure 1: Oxidative stress cycle.

Hyaluronic acid role in osteoarthritis

Hyaluronic acid, a non-sulfated glycosaminoglycan, consists of alternating repeating D-glucuronic acid and N-acetylglucosamine units. It is a major component of synovial fluid and is mainly produced by type B synoviocytes. Its hydrophilic properties allow for high solubility in aqueous environments, ensuring tissue hydration [15]; this quality helps maintain the articular matrix's viscoelastic structure by providing joint lubrification and shock absorption [16]. Other critical functional properties of HA are anabolic by the stimulation of glycosaminoglycans and type II collagen formation [17], anti-inflammatory by decreasing ROS, and analgesic by inhibiting tissue nociceptors, and pain mediator substance PY [16].

During OA, inflammatory cytokines such as interleukin-1b promote joint and chondrocyte degeneration by deregulation and loss of essential macromolecules from the ECM such as type II collagen and proteoglycan biosynthesis; thus, OA is characterized by a considerable reduction of hyaluronate concentration leading to pain and loss of articular homeostasis. Furthermore, in experimental models, the administration of exogenous HA provided a decrease in ROS synthesis, and thus, a reversion of the inhibition of proteoglycan synthesis caused by both the inflammatory process and mechanical overload stress [18]. In human OA, intra-articular injection of HA provided a significant reduction in synovial fluid levels of peroxide hydrogen (H2O2) and oxygen species, suppressing the H2O2 induced cell death [19]. In addition, it also prevents IL-1β-induced oxidative stress, along with a decrease in inflammatory species like metalloproteases, nitric oxide, and prostaglandins [20]. By this means, intraarticular visco-supplementation restores articular viscoelastic properties by exerting chondroprotective and anti-inflammatory effects (Table 1).

Table 1: Therapeutic benefits of viscosupplementation.

Therapeutic Benefits of Viscosupplementation
Stimulation of Metabolism
Prevention of apoptosis of chondrocytes
Inhibition of Chondral degradation and articular inflammatory responses
Decrease of lymphocytes proliferation

There are several preparations of HA described in the literature. Regarding molecular weight. A low molecular weight (0.5-1.5 million Dalton) can achieve maximum HA joint concentrations to reduce inflammation, but it presents a low elastoviscosity needing at least four to five injections to achieve its effectiveness [21]. On the other hand, the high molecular weight preparation (6-7 million Dalton) shows better joint fluid retention and a superior anti-inflammatory effect. To increase HA molecular weight and provide persistent activity a cross-linking technique has been developed. Controlled cross-linking produces a viscous gel with increased density and viscoelasticity requiring only one injection. This single-injection regimen is attractive as it decreases patient time expenditure and infiltration discomfort [21]; but, on the other hand, there are also higher side effects reported with cross-linked HA, especially synovitis.

However, until now, current evidence does not support the superiority of one kind of HA preparation over another except perhaps, for a slightly lower efficacy for low weight HA preparations versus intermediate and high weight hyaluronic acid [22,23] as well as an increased safety risk for high weight – HA. In some studies even doubling the frequency of post-injection effusion and inflammation [22,24]. Therefore, in our practice, we use medium-plus low weight hyaluronic acid; the background behind this choice is that the mixing of the two hyaluronates allows a high concentration of HA (2,5%), maintaining a viscosity suitable enough for supplementation; easy to handle and with an optimum extrusion from the syringe, but without the adverse inflammatory effects of low or high weight HA [25].

Hyaluronic acid stabilization agents

Despite good outcomes described for HA, clinical trials provide controversial results regarding its effectiveness [26,27]; there are several possible explanations for these conflicting results. Discrepancies may originate from differences in study design, outcome measures, and differences in the composition between the products that vary widely in their concentration, molecular weight, and molecular cross-linkage.

One of the weaknesses of exogenous HA is its rapid clearance following intra-articular injection, thus, limiting the time of intraarticular residence from about one week [28,29] for the cross-linked solutions to only a few days in the best scenario for linear molecules [29]. In this clearance process, ROS plays a critical role among the multiple mechanisms contributing to HA degradation.

In an attempt to optimize hyaluronic acid's effectiveness and intraarticular half-life, different products have been investigated, for example, sugars [30,31], polyols [32,33], amino acids [34,35], and salts. These stabilizers provide a safe and non-invasive approach in which their function is to preserve the covalent architecture of protein. Exhaustive experimentation with polyol enhanced HA stabilization has been made with promising results [20,36]. In an in vitro study with HA-Sorbitol, it was proved to prevent IL-1b induced oxidative stress, measured by ROS species, and NADPH oxidase phosphorylation, as well as metalloproteinases, nitric oxide, and prostaglandin release; thus, supporting the evidence of its properties of redox restoration in OA [20].

Conrozier et al., in an in-vitro study, demonstrated that mannitol enhanced hyaluronic acid protects hyaluronate from ROS-mediated degradation compared to standard therapy; the two HA not containing mannitol were rapidly degraded by H2O2, as demonstrated by the remarkable decrease of the complex viscosity. On the other hand, the rheological properties (behavior of flow deformation and its internal structure) of mannitol-HA were not substantially modified in the presence of H2O2. Therefore, mannitol might increase HA intra-articular residence time without significantly modifying its rheological behavior [37]. Devi et al. In a study where sorbitol, xylitol, Myo-inositol, and trehalose disaccharide were compared in retaining lysozyme enzyme activity when incubated at 60ºC, for 24 hours; Myo-inositol, as well as trehalose, had the best action retaining 72% of activity at 0.75M, and 1.5M concentration respectively at a PH of 7. Interestingly the addition of guanidine chloride denaturant at a low concentration (1M) increased the activity retention to 98% for both products[38].

Due to the multifactorial etiology of OA, in vivo study results are less significant; the most studied antioxidants are mannitol and sorbitol [39-41]. Unfortunately, most of the in vivo studies are not controlled [39-42], or observational [40,41], with a low number of patients [39,43], and without evident superior outcomes over controls [36]. Thus, until now, there is no strong in vivo evidence to recommend their use, and still larger multicentric studies are needed along with standardization of regimen doses and ideal patient characterization for best outcome measure.

Trehalose disaccharide

Trehalose is a nonreducing disaccharide in which two glucose molecules are linked together in an α,α-1,1 linkage (Figure 2). This disaccharide is widespread throughout the biological world. It can be found in plants, yeast, fungi, bacteria, and insects. Although the complete function of trehalose in nature is not fully understood, plants produce higher homologs of sucrose such as raffinose (Gala1-6Sucrose) and stachyose (Gala1-6Gala1-6Sucrose); these higher sucrose oligosaccharides have been proposed to play a role in stabilizing or protecting cells against stress [44].

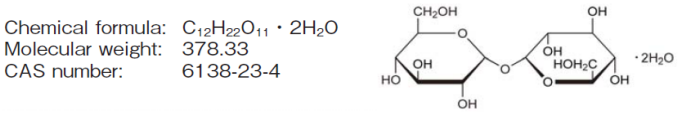

Figure 2: Trehalose chemical formula.

Human beings cannot produce trehalose naturally; thus, it has to be externally administrated. However, it has been widely used in many forms and pharmaceuticals as Food and Drug Administration (FDA) approved excipient [44,45]. Trehalose glycosidic α,α-1,1 bond confers some of its remarkable properties, like the ability to remain stable to heat and acid. Furthermore, its low hygroscopicity property allows it to remain stable and free-flowing at high humidity environments like the knee joint [44] (Table 2).

Table 2: Trehalose characteristics.

Trehalose Characteristics
Non reducing disaccharide
Relatively inert glycosidic linkage (non-reactivity protects product)
Absence of internal hydrogen bonds
High glass transition temperature 115ºC (Stable amorphous state)
Hight hydrophilicity and hydration number
Decrease in lipid melting temperature
Prevention of protein structure denaturation
Low hygroscopicity (no moisture absorption)
Stable to heat and acid (PH2 at 100º C for 24h)

Trehalose benefits as a stabilizing agent have been widely studied. Protein-based compounds such as hyaluronic acid are easily degraded by strong acids, alkaline enzymes, inorganic salts, and organic solvents. The active end groups of these proteins, generally associated with hydrogen-bonded water, can bind to other molecules, leading to its denaturation and loss of function. Trehalose helps to preserve the structure and function of proteins by forming hydrogen bonds with the polar residues in hyaluronic acid, functioning as a water substitute. In this setting, the removal of H_2O prevents the occurrence of chemical reactions, thus preventing the denaturation of the hyaluronate structure.

Trehalose has not only been shown to stabilize enzymatic reactions but also to have anti-inflammatory properties. In addition, it serves as an autophagy activator in many cells through the mTOR (Mammalian Target of Rapamycin) independent pathway [46,47]. Thus, trehalose exerts cell-protective effects under various stress conditions such as oxidative damage, dehydration, and temperature changes. Potential mechanisms of trehalose-induced anti-apoptosis effects include rescuing mitochondria dysfunction via BNP3 upregulation (Bcl-2 nineteen-kilodalton interacting protein 3), a protein responsible for autophagy mechanism, thus suppressing endoplasmic reticulum (ER) stress and restoring autophagic flux [48]. By the exact mechanism of BNP3 regulation, trehalose has been shown to reverse age-related arterial stiffening, which could be directly related to OA senescence pathogenicity as well as a metabolic-inflammatory syndrome [46,48]. Other uses of trehalose as a human anti-inflammatory and stabilizing agent are in organ preservation to increase organ transplantation timing; and as a reducer of postoperative adhesions for fibrosis prevention [49,50]. Thus, these anti-inflammatory properties could be the cornerstone in OA disease prevention and slowing down its progression.

Trehalose in osteoarthritis

The protective effects of trehalose have been reported in several degenerative diseases, but there is not much described for its application in OA disease. In a mouse model of OA Tang et al. investigated trehalose's effects and therapeutic potential on apoptosis and autophagy in chondrocytes under oxidative stress. Oxidative mediated chondrocyte apoptosis, measured by an increase of caspase 3, correlated with a decrease of autophagy, measured with an increment of p62 protein. Furthermore, when trehalose was added, it proved to restore oxidative stress and autophagic flux disruption, with an upregulation of proteins (SQSTM1/p62) involved in mitochondria stress-related apoptosis pathway [51].

Regarding hyaluronic acid stabilization, a recent in vitro study showed that hyaluronic acid combined with trehalose had improved resistance to hyaluronidase enzyme degradation compared to non-trehalose-HA [52]. In this study, a hyaluronate composed of 80% medium weight; plus 20% low weight hyaluronic acid was compared to one with the same structure plus a 1% trehalose. The two samples were tested in a reactive solution with an added hyaluronidase for its activity evaluation. The sample containing trehalose showed no significant amount of hydrolyzed hyaluronic acid in the reaction mix after 60 minutes, while in the control solution, 54.1% of HA was hydrolyzed (*P<0.05)*, meaning a lower susceptibility and accessibility of the product to the hyaluronidase than the sample without trehalose [53,54].

Provided this evidence, we developed a prospective randomized, double-blinded, controlled trial comparing 1% trehalose hyaluronic acid (T-HA) versus non-trehalose hyaluronic acid, administered as a three-dose infiltrative therapy, separated by 15 days. Selected patients were persistent symptomatic OA knees, Kelgreen-Lawrence grade II-IV, without pain relief for three months after non-invasive treatment. KOOS, IKDC, and VAS clinical scores were compared at three and six months from the basal score. A sample of 60 patients was previously calculated for a study power of 0.80. The mean age was 56,4 ± 15,6 DS. At three months, IKDC, KOOS, and VAS score improved for both groups (*P* <0.05), but there were no significant differences at three months between the two treatments. Interestingly enough, at six months, scores continue to improve in the T-HA, while in the control group, scores decreased to the baseline; this difference was significant (*P*<0.05) (Fig, 3-5). Thus, according to in vitro studies and our findings, we could conclude that trehalose hyaluronic acid was safe and provided pain relief in persistent osteoarthritis knee symptoms for at least six months, lasting longer than non-trehalose hyaluronic acid. However, it is essential to remember that these are initial studies and that multicentric studies with a longer duration will be crucial for determining the potential of trehalose treatment for osteoarthritis treatment.

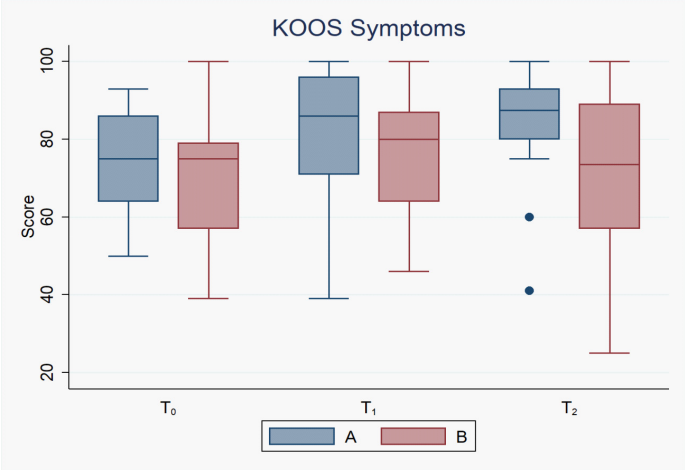

Figure 3: Box-Plot KOOS symptoms.

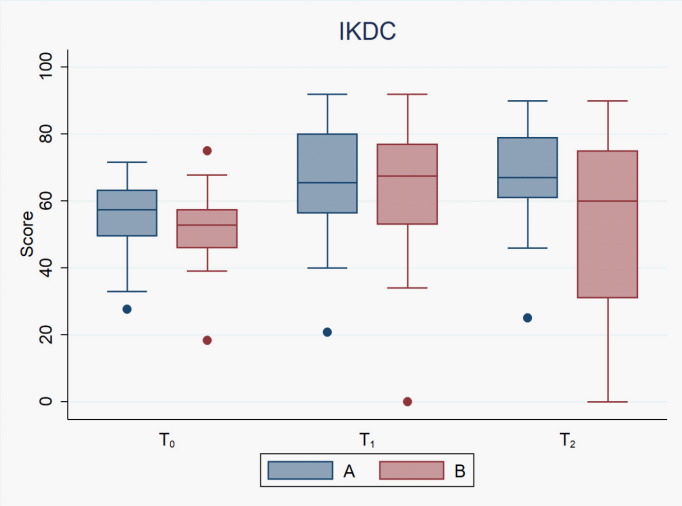

Figure 4: Box-Plor IKDC score.

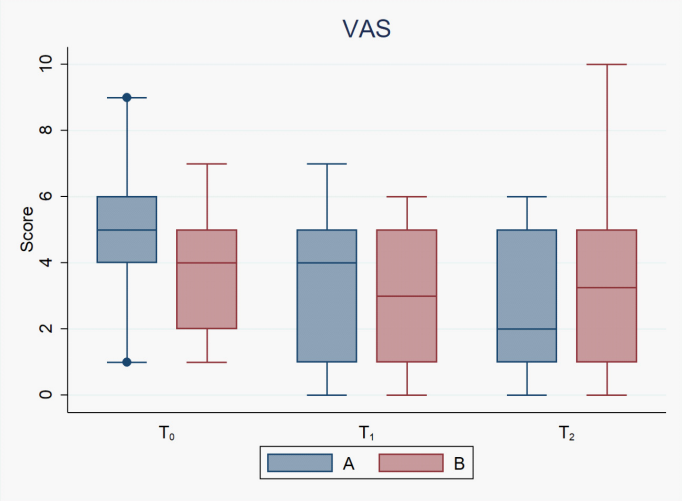

Figure 5: Box-Plot VAS score.

Conclusions and key points

- ✓ Osteoarthritis is the most prevalent joint degenerative disease, and it is increasing at an exponential rate with high social and economic costs.
- ✓ Along with increasing knowledge of the inflammatory pathology pathway of OA, newer, less invasive therapies are being developed for the prevention and early treatment of OA.
- ✓ Hyaluronic acid has long ago been used because of its important properties in stopping ROS inflammatory pathways, with good results in vitro but contradictory results in vivo.
- ✓ As an attempt to stabilize hyaluronic acid action, trehalose has appeared as a new and safe alternative that has upgraded its effects by providing longer-lasting results and potentiating the anti-inflammatory effects.
- ✓ Lastly, it is essential to recall that every patient with OA syndrome must be first studied, and the primary cause of OA must be ruled out. Therefore, the essential treatment should be directed to the primary causal factor, and intra-articular infiltrative therapy may be a coadjuvant treatment option in persistent cases for slowing OA progression and pain control.

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