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Evaluation of the Anti-inflammatory Effect of Intra-articular Injection of Chondroitin Sulfate and Sodium Hyaluronate in Mechanically Induced Temporomandibular Joint Injury in Rabbits

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Abstract

BACKGROUND: Degenerative arthritis is the most common form of arthritis, usually affecting the hands, feet, spine, knees, and temporomandibular joint (TMJ) as well. TMJ degenerative arthritis causes symptoms of painful joints, loss of joint function, limited mouth opening, joint instability, and clicking. Non-surgical symptomatic treatments can successfully be used to treat patients with degenerative arthritis.

AIM: This study aimed to evaluate the anti-inflammatory effect of intra-articular injection of chondroitin sulfate and sodium hyaluronate in mechanically induced acute injury in TMJ of rabbits.

METHODS: An animal study was conducted, all rabbits received a mechanical injury using a contra-angle handpiece with a speed of 120 rpm of a fissure bur 4 mm in diameter and 4 mm in depth extending to subchondral bone. Thirty-two rabbits were randomized into four groups: Control group, sodium hyaluronate "SH" group, chondroitin sulfate "CS" group, and "CS-SH" group. After 7 days, rabbits in the control group, "SH" group, "CS" group, and "CS-SH" group were respectively treated with normal saline, sodium hyaluronate, chondroitin sulfate, or combination of CS&SH injection in the TMJ. All animals were treated once weekly for 3 weeks. A histological evaluation was performed.

RESULTS: Histological findings showed a significantly reduced inflammatory cell, bone resorption, and fibrosis in the CS-HA-treated group.

CONCLUSION: CS-SH injection has an anti-inflammatory effect on TMJ degenerative arthritis and aids in the reparative process.

Introduction

Degenerative arthritis is a type of arthritis caused by inflammation, breakdown, and degeneration of the cartilage of the joints. It is the most common form of arthritis, usually affecting the hands, feet, spine, knees, and temporomandibular joint (TMJ) as well, it is also known as degenerative joint disease [1]. TMJ degenerative arthritis affects the cartilage, subchondral bone, synovial membrane, and other hard and soft tissues causing changes such as TMJ remodeling, articular cartilage deterioration, abrasion, and local thickening and remodeling of the underlying bone [2]. Management of degenerative arthritis is largely symptomatic. Studies have shown that non-surgical treatment can successfully be used to treat patients with osteoarthritis [3], [4], [5].

Hyaluronic acid is a polysaccharide, it is the main component of the cartilage and the synovial fluid; it is responsible for the mechanical properties of the joint by allowing shock absorption, cartilage protection, and lubrication [6]. In osteoarthritis patients, synovial hyaluronate is depolymerized and is cleared at higher rates compared to normal subjects due to inflammation [7]. Intra-articular HA injection is an effective tool in reducing the pain and symptoms associated with internal derangement of TMJ [8].

Chondroitin sulfate – a sulfated glycosaminoglycan – is an important structural component of the extracellular cartilage matrix. It is an inhibitor of extracellular proteases involved in the metabolism of connective tissues and stimulates proteoglycan production by chondrocytes *in vitro*; it also inhibits cartilage cytokine production and increases the intrinsic viscosity of the synovial liquid [9]. Some authors found that intra-articular injection of chondroitin sulfate stimulated the chondrocyte metabolic activity and was possibly helpful to decrease the degenerative process [10], [11].

Preliminary clinical trials were in favor of the effectiveness of intra-articular injection of sodium hyaluronate combined with chondroitin sulfate as a viscosupplementation for the degenerative osteoarthritic TMJ and this combination can be used as a safe and effective treatment for all cartilage lesions [10], [12].

Materials and Methods

An animal study was conducted in the following design.

Animals

All experimental procedures were approved by the Research Ethics Committee of the Faculty of Dentistry, Suez Canal University (Ismailia, Egypt), (I.R.B. no. 76/2018) and provided ethical guidelines for the study.

The sample size was based on a previously published study with a similar experimental design [13].

Thirty-two mature (aged 6 months or more) male New Zealand rabbits were employed in this study. They were selected according to weight (2.5−3 kg). Rabbits were housed in clean well-ventilated stainless steel cages, at temperature of 25 ± 3°C throughout the experiment and were left 1 week for acclimatization, no special feeding were provided other than the known protocol in the animal house at the Faculty of Medicine – Suez Canal University.

Drugs and chemicals used

- Sodium hyaluronate in the form of HYALGAN 20 mg/2 ml syringe, Fidia Farmaceutici S.p.A. Italy.
- Chondroitin sulfate in the form of ampules of 200 mg/20 ml of chondroitin sulfate, Nichi-Iko Pharmaceutical Co., Ltd., Japan.

Induction of full-thickness osteochondral defect

Full-thickness osteochondral defect – to simulate osteoarthritic changes – was created in the left TMJ of all rabbits by the following technique: [12], [14].

- General anesthesia was induced with an intramuscular injection of 50 mg/kg ketamine HCl (ketamine HCl 200 mg/mL, injectable solution, 10 mL, by NexGen pharmaceuticals Co., NY, USA) and was maintained with an intramuscular injection of 5 mg/kg xylazine HCl (Xylazine HCl 125 mg/mL, injectable solution, 100 mL, by NexGen Pharmaceuticals Co., NY, USA) and 0.3 mg/kg ketamine HCl.
- One side temple area of the rabbits was shaved, scrubbed with 10% povidone iodine, and draped in a sterile fashion.
- The left TMJ area incision was performed (Figure 1).

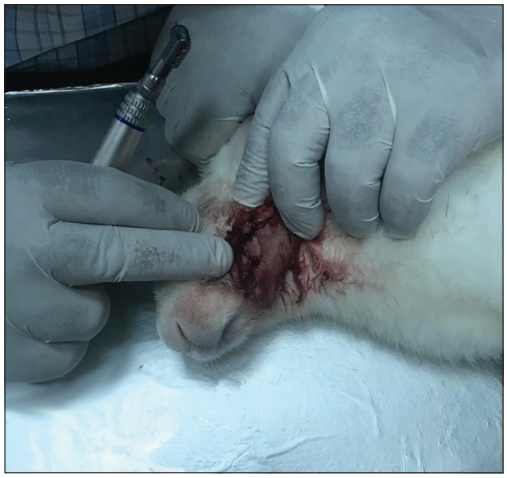

Figure 1: Flap incision at the left side temple of the rabbit to expose the TMJ area

- A full-thickness osteochondral defect using electric micromotor contra-angle handpiece with a speed of 120 rpm of a fissure bur 3–4 mm in diameter and 4 mm in depth extending to subchondral bone in TMJ condyles was then created (Figure 2a and b).

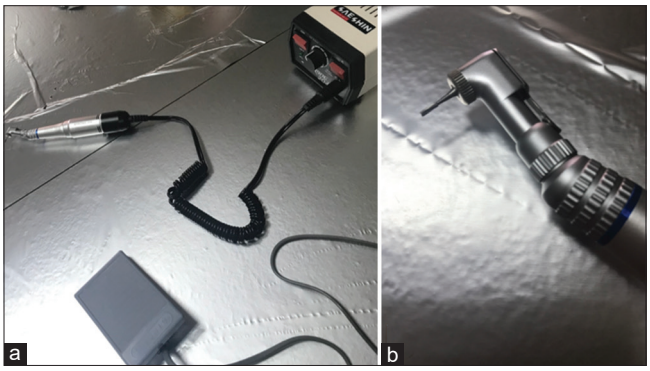

Figure 2: (a) The electric micromotor and (b) The contra-angle handpiece with a fissure bur

- Flap was closed using absorbable suture material, then animals were left for 1 week for complete soft-tissue healing.

Experimental study design

- Animals were divided into four groups, each group consisted of eight rabbits.
- Group (1): Saline control group; 0.1 ml saline – to simulate no treatment – was injected intra-articular in TMJ once per week for 3 weeks.
- Group (2): Hyaluronic acid-treated group; 0.1 ml hyaluronic acid (16 mg/mL) was

injected intra-articular in TMJ once per week for 3 weeks, according to Tosun *et al*. [12].

- Group (3): Chondroitin sulfate-treated group; 0.1 ml CS (20 mg/mL) was injected intra-articular in TMJ once per week for 3 weeks.
- Group (4): Hyaluronic acid + chondroitin sulfate treated group; 0.1 ml HA (16 mg/mL) + CS (20 mg/mL) were injected intra-articular in TMJ once per week for 3 weeks [12].

Histopathological sample preparation

On the 6th week post-injection [14]; to insure reasonable cartilage and bone healing; rabbits in all groups were euthanized by over dose of ketamine HCl injection. Osteochondral tissues were separated and fixed in a 10% phosphate-buffered paraformaldehyde solution. Tissues were dehydrated, embedded in paraffin, and sectioned at 4 μm and stained with hematoxylin and eosin (H&E) (Life Chemicals group, Alexandria, Egypt). After sacrificing and taking the specimens, the dead experimental animals were disposed of by burning in the Animal Ashing Unit of the Faculty of Medicine – Suez Canal University. Then, specimens were examined blindly under a light microscope to evaluate the severity of osteochondral defect, condylar cartilage tissue, and subchondral bone.

Results

Rabbits with full-thickness osteochondral defect of control group that received normal saline showed marked inflammatory infiltrate formed of lymphocytes and many plasma cells associated with marked fibrosing reaction, bone resorption, few degenerated osteocytes, and few hyperplastic osteoblasts and scattered osteoclasts. These deleterious effects associated with full-thickness osteochondral defect were attenuated in severity with hyaluronic acid and/or chondroitin sulfate administration. However, treatment with combined hyaluronic acid and chondroitin sulfate was more beneficial in attenuating the severity of osteochondral defect with minimal to no residual inflammation, minimal fibrosis, and osteoblastic rimming compared to the control group (Figure 3a-d and Table 1).

I-B-Statistical analysis

Inflammatory cells

Inflammatory cells were present in 100% in control group, in 62.5% in sodium hyaluronate group, in 25% in chondroitin sulfate group, and in 12.5% in sodium hyaluronate and chondroitin sulfate group. This difference between groups was statistically significant (p = 0.001) (Figure 4).

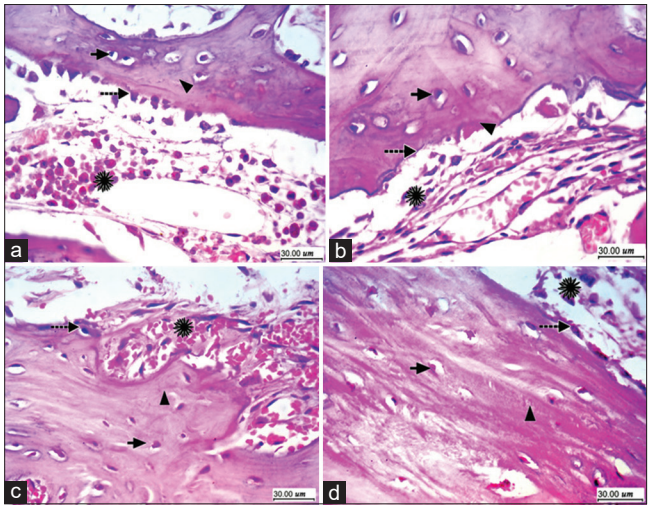

Figure 3: The histopathological picture of the TMJ tissues from the experimental groups (H&E X400). (a) Rabbits with full-thickness osteochondral defect that received normal saline showed bone tissue surrounded by marked inflammatory infiltrate formed of lymphocyte and plasma cell and marked fibrosing reaction within medullary spaces (asterisk). There is evidence of bone resorption with irregular woven bone (arrowhead) and few degenerated osteocytes (arrow). There are few hyperplastic osteoblasts were observed (dashed arrow). (b) Hyaluronic acid administration induced reduction in inflammatory cells infiltration (asterisk). Bone tissue (woven and mature) showed bone matrix with focal bone resorption (arrowhead) and regularly arranged osteocytes (arrow). Surrounding tissue showed organization and healing with focal fibrosis. Few degenerated osteoblasts were observed (dashed arrow). Chondroitin sulfate group (c) revealed residual moderate to marked inflammatory cells infiltration within marrow spaces and in surrounding tissues (asterisk). Bone tissue (woven and mature) showed bone matrix with almost no bone resorption (arrowhead) with regularly arranged osteocytes (arrow). Surrounding tissue showed residual focal congestion and few degenerated osteoblasts (dashed arrow). Hyaluronic acid and chondroitin sulfate group (d) revealed minimal to no residual inflammation in the form of very few scattered inflammatory cells within marrow spaces and in surrounding tissues with minimal fibrosis (asterisk). Bone tissue (woven and mature) showed bone matrix with almost no bone resorption (arrowhead) with regularly arranged osteocytes (arrow). Osteoblastic rimming was observed (dashed arrow)

Table 1: Comparison of histological findings in different groups

Group	Inflammatory cells (%)		Bone resorption (%)		Fibrosis (%)		Osteoclasts (%)		Osteoblasts (%)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Control group (saline)	8 (100)	0	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	3 (37.5)	5 (62.5)
Sodium hyaluronate group	5 (62.5)	3 (37.5)	5 (62.5)	3 (37.5)	5 (62.5)	3 (37.5)	6 (75)	2 (25)	5 (62.5)	3 (37.5)
Chondroitin sulfate group	2 (25)	6 (75)	3 (37.5)	5 (62.5)	3 (37.5)	5 (62.5)	2 (25)	6 (75)	6 (75)	2 (25)
Sodium hyaluronate and chondroitin sulfate group	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)	7 (87.5)	1 (12.5)
χ^2	10.5		6.5		6.5		8.5		7.3	
p	0.015*		0.089 (NS)		0.089 (NS)		0.037*		0.088	

Chi-square test, significance level p ≤ 0.05, *significant. NS: Non-significant.

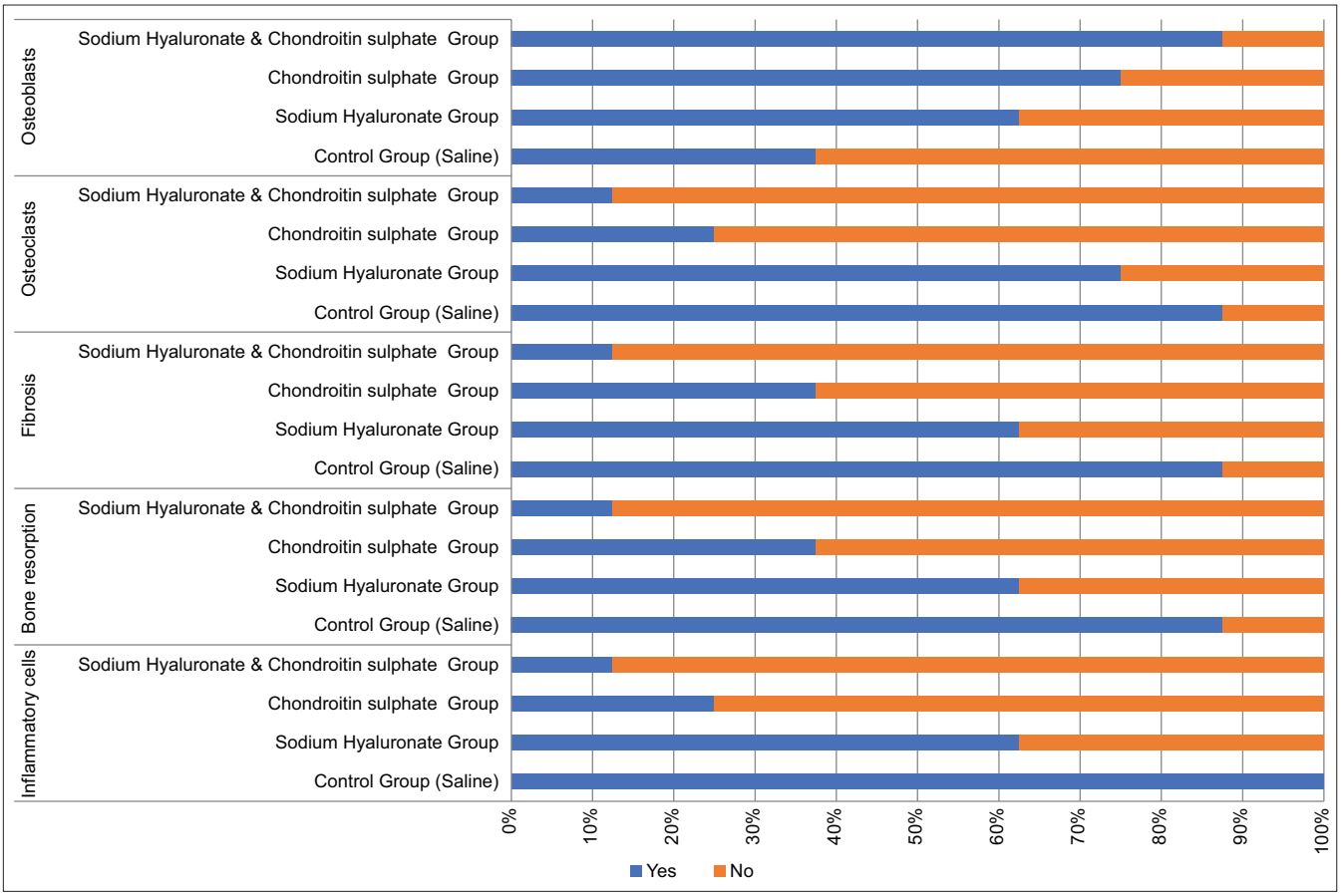

Figure 4: Bar chart illustrating frequency of histological findings in different groups

Bone resorption

Bone resorption was present in 87.5% in control group, in 62.5% in sodium hyaluronate group, in 37.5% in chondroitin sulfate group, and in 12.5% in sodium hyaluronate and chondroitin sulfate group. This difference between groups was statistically significant (p = 0.019).

Fibrosis

Fibrosis was present in 87.5% in control group, in 62.5% in sodium hyaluronate group, in 37.5% in chondroitin sulfate group, and in 12.5% in sodium hyaluronate and chondroitin sulfate group. This difference between groups was statistically significant (p = 0.019).

Osteoclasts

Osteoclasts were present in 87.5% in control group, in 75% in sodium hyaluronate group, in 25% in chondroitin sulfate group, and in 12.5% in sodium hyaluronate and chondroitin sulfate group. This difference between groups was statistically significant (p = 0.007).

Osteoblasts

Osteoblasts were present in 37.5% in control group, in 62.5% in sodium hyaluronate group, in 75% in chondroitin sulfate group, and in 87.5% in sodium hyaluronate and chondroitin sulfate group. This difference between groups was not statistically significant (p = 0.259).

Discussion

The experimental study was based on 32 male rabbits that were subjected to induction of osteochondral defect, we were investigating the effect of intra-articular injection of sodium hyaluronate + chondroitin sulfate on inflammatory reaction and cartilage formation on the defect, through histopathological observation. Many osteoarthritic animal models used surgical approaches to initiate joint degeneration, and each method is designed to study a specific aspect of the injury or subsequent disease development, the present experimental study design was in accordance with multiple researches in terms of creating mechanical osteochondral defect in animals, either in dogs [11], sheep [15], or in

rabbits [13], [16], [17], [18] as in this case, we selected New Zealand rabbits for being an easily available and less aggressive animals [19], [20], although the horse articular cartilage is the most comparable to humans, and they have been used to study articular cartilage repair and osteochondral defects [21], [22], yet rabbits remain easier for manipulation and have affordable cost.

In all groups, there was an inflammatory infiltrate formed of lymphocytes and many plasma cells associated with marked fibrosing reaction, bone resorption, few degenerated osteocytes, and few hyperplastic osteoblasts with scattered osteoclasts, nevertheless, these deleterious effects associated with full-thickness osteochondral defect were attenuated in severity with hyaluronic acid and/or chondroitin sulfate administration.

In hyaluronic acid and chondroitin sulfate sections, there was minimal to no residual inflammation in the form of very few scattered inflammatory cells within marrow spaces and in surrounding tissues with minimal fibrosis and the inflammatory cells had a statistically significant difference compared to other groups ($p = 0.001$), these results go along with multiple findings of previous researches that proved that CS and/or the sulfated disaccharides appear to elicit an anti-inflammatory effect at the synovial membrane and chondrocytes levels [23], [24], as explained by Omata *et al*. [25] that chondroitin had a biological effect on animal models through significant inhibition of osteoarthritic edema, synovitis, and destruction of the articular cartilage as well as reduced CRP and IL-6. Moreover, Bauerova *et al*. [26] proved that CS reduced the production of pro-inflammatory cytokines, CRP, phagocytic activity, and the intracellular oxidative burst of neutrophils.

In vivo in different experimental arthritis, the number and severity of articular symptoms decrease after CS administration. In bones, CS accelerates the mineralization process and bone repair, which agrees with the present results. For new bone tissue (woven and mature), present results showed bone matrix with almost no bone resorption with regularly arranged osteocytes and few regenerated osteoblasts as well as normal cartilage mineralization (calcified cartilage) which was found to be higher in the HA+CS group compared to the HA, CS, and control groups, although the difference was not statistically significant, it might need longer time to show these changes in bone. Several recent studies have shown almost similar results [13], [18].

Previous animal model-based studies have examined the beneficial effect of CS intra-articular injection in degenerate cartilage, their results reflected the presence of less obvious degenerative injuries, assuming that chondroitin sulfate stimulated the reparative process or delayed the disease evolution [11], [27]. Moreover, sodium hyaluronate has a viscosupplementation effect when used by intra-articular access, which improves the viscosity of the synovial fluid, decreases attritions and erosions, and also decreases the impact on the injured cartilage [28]. On the other hand, Smith *et al.* [29] did not observe any improvement in the cartilage morphology when he used sodium hyaluronate as intraarticular injection.

These overall findings coincide with the present results, as they showed that inflammatory cells and osteoclastic activities were minimal in the group that received both SH and CS. On the other hand, fibrosis, bone resorption, and osteoblasts findings among groups did not show any significant difference.

Conclusion

This study shows that intra-articular injection of a combination of chondroitin sulfate and sodium hyaluronate has an anti-inflammatory effect on the degenerative osteoarthritis of joints and it aids in the reparative process as well, as shown histopathologically.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

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