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 CS-SH injection group compared to other groups. Histological changes in the CS-SH group were less pathological changes compared to other groups.

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. 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 fluid [9]. Some authors found that intra-articular injection of chondroitin sulfate stimulates 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 0.3 mg/kg xylazine 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).

**Table 1: Comparison of histological findings in different groups**

| Group                        | Inflammatory cells (%) | Bone resorption (%) | Fibrosis (%) | Osteoblasts (%) | Osteoclasts (%) |
|------------------------------|------------------------|---------------------|--------------|-----------------|-----------------|
|                              | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| Control group (saline)       | 8   | 100| 7   | 12.5| 7   | 12.5| 7   | 12.5| 7   | 12.5|
| Sodium hyaluronate group     | 5   | 62.5| 3   | 37.5| 5   | 62.5| 3   | 37.5| 5   | 62.5|
| Chondroitin sulfate group    | 2   | 25 | 6   | 75 | 3   | 37.5| 3   | 37.5| 5   | 62.5|
| Sodium hyaluronate and CS    | 1   | 12.5| 7   | 87.5| 1   | 12.5| 7   | 87.5| 1   | 12.5|
|                            | 10.5 | 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 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).

**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).
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 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 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 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...
rabb的目标，我们选择了新西兰兔子，因为它们容易操作且成本实惠 [19, 20]，尽管马关节软骨的组织结构与人类最为相似，它们已经被用来研究关节软骨修复和骨软骨病损 [21, 22]，然而兔子的治愈过程仍然更容易，且成本较低。

在所有组中，存在炎症细胞的浸润，形成淋巴细胞和许多浆细胞，并与标记的纤维化反应，骨吸收，未分化骨细胞，骨细胞，和稀疏的修复成骨细胞，骨吸收，未成熟骨细胞，骨细胞，以及在周围组织中弥漫的炎症细胞，结果表明，与HA，CS，和对照组相比，HA+CS组的炎症细胞数量和严重程度下降，显示了骨质破坏的减少，某些骨细胞和骨细胞活动的减少。

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结论

这项研究证明，关节软骨硫酸和硫酸软骨素对骨软骨病损具有抗炎作用，对骨软骨化作用以及骨软骨病损有保护作用。

数据可用性

这些结果与之前的动物模型实验一致，如Bauerova et al. [26]证明，CS在动物模型中通过显著抑制炎性细胞和减少CRP和IL-6，对炎性细胞有抗炎作用。

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