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Case Report

Adelmidrol Protects Hyaluronic Acid against Oxidative Degradation and Improves the Outcome in Patients with Adhesive Capsulitis of the Shoulder Managed by Physical Therapy. *In vitro* Evidence and Two Case Reports

Cavallaro Carlo [1*], Da Roit Marco[2], Marcolongo Gabriele[3], Greggio Francesco[3], Gugliandolo Enrico[4], Crupi Rosalia[4], Cuzzocrea Salvatore[5]

[1]Functional Recovery and Rehabilitation Unit, San Martino Hospital, AULSS1 Dolomiti Belluno, Italy

[2]Belluno Comunity Hospital, AULSS1 Dolomiti - Belluno, Italy

[3]Epitech Group Chemical Research Laboratories, Saccolongo, Padova, Italy

[4]Department of Veterinary Science, University of Messina, Messina, Italy

[5]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy

***Corresponding author:** Carlo Cavallaro, Functional Recovery and Rehabilitation Unit, San Martino Hospital, AULSS1 Dolomiti Belluno, Italy

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Abstract

Adhesive capsulitis (AC) or frozen shoulder is an inflammatory condition characterized by joint stiffness and pain. Although its pathogenesis is not fully elucidated, inflammation (either acute or low-grade inflammation) is believed to play a crucial role. An increase in reactive oxygen and nitrogen species also occurs in AC. The management of AC is challenging for orthopedic surgeons and physical therapists. Intra-articular hyaluronate injection is a treatment option. Yet, failure is reported and may depend upon oxidative breakdown of hyaluronan. Adelmidrol is a palmitoylethanolamide analogue and azelaic acid derivative exerting antioxidant activity in a number of fibrotic disorders. Here we first report the *in vitro* ability of Adelmidrol to protect hyaluronan against oxidative degradation and to exert an antioxidant effect against hydrogen peroxide-induced increase in nitrite release and intracellular reactive oxygen species in the macrophage J774A.1 cell line. We then investigated a new intra-articular formulation of high molecular weight (1300-2000 kDa) hyaluronic acid (1%) and Adelmidrol (2%) in two AC patients partially responding to physical therapy. Within a tailored physical therapy approach, three fortnightly intra-articular injections of this formulation allowed for a progressive reduction in pain severity, as well as improved range of motion and daily living activities, with the good outcome being maintained at the six-month follow-up in both patients. Overall, the data show Adelmidrol's protective activity against hyaluronic acid oxidation and provide preliminary information for further studies to investigate the association of Adelmidrol and high molecular weight hyaluronic acid in the management of AC patients.

Keywords: Hyaluronic acid; Adelmidrol; Adhesive capsulitis; Intra-articular injections; antioxidant; shoulder

Introduction

Adhesive capsulitis of the glenohumeral joint (AC), also referred to as frozen shoulder, represents one of the most disabling arthropathies of the shoulder [1]. Joint pain and functional disability severely impact the patient's physical and psychological health, as well as social well-being [2]. Based on etiology, AC can be classified as primary (idiopathic and usually with a gradual onset) or secondary (e.g., resulting from shoulder trauma or surgery) [2]. Although AC pathogenesis is not fully elucidated, intra-articular inflammation and the following reactive fibrosis are the main features, progressively leading to stiffness and reduced range of motion [3]. Like degenerative joint disorders (i.e., osteoarthritis) [4], AC is featured by an increase of immune-inflammatory cells within the joint, including mast cells and macrophages [5,6]. If inadequately controlled by pro-resolving responses, the excessive intra-articular release of immune cell mediators (i.e., cytokines, growth factors, and lytic enzymes) may drive inflammation and fibrosis at the synovial and whole joint level [6-8]. Oxidative stress has long been recognized as a key player in a number of musculoskeletal diseases [9] and is currently considered an important factor in AC pathogenesis [10]. The excessive generation of reactive oxygen and nitrogen species is thought to be responsible not only for cellular damage, but also for initiating the oxidative degradation of biomolecules, like the polysaccharide hyaluronic acid (HA) [11-13]. Indeed, higher levels of low molecular weight HA have been observed in diseased compared to healthy joints [14,15]. It is thus conceivable that, upon injection in the oxidative environment of AC shoulders, HA would be degraded into smaller fragments with negative consequences on intrinsic viscosity and lubricating properties [16].

This may account for the limited treatment effect of HA intra-articular injections in AC patients, mainly with respect to the short duration and pain relief [17,18]. Although self-limiting, AC generally resolves within 18 to 30 months [2,19]. The AC patient experiences a long condition of severe functional limitation and pain, accordingly. Indeed, current medication with nonsteroidal anti-inflammatory drugs (oral) and corticosteroids (either oral or intra-articular injections), provide pain relief in the short-term, while long-term outcomes are still unsatisfactory [20]. Oxidant-protected HA may thus be of help, similar to what was previously suggested for rheumatoid arthritis [21]. Adelmidrol (ADM) is the international nonproprietary name of an azelaic acid derivate and an analogue of palmitoylethanolamide (PEA) [22]. PEA is a naturally occurring lipid amide exerting a local, autacoid, anti-injury function via mast cell down-modulation, as originally speculated by the Nobel laureate Rita Levi Montalcini [23] and later repeatedly confirmed [24-27]. ADM was shown to increase the endogenous levels of PEA [28], the latter being reduced in the course of joint diseases [29]. Recent evidence supports the *in vivo* antioxidant activity of ADM in animal models of colitis [30], ischemia/reperfusion injury [31] and pulmonary fibrosis [32], but the antioxidant protective effect has not yet been investigated at the molecular and cellular level. Recent preclinical and clinical studies have shed some light on the benefit of a new intra-articular formulation based on high molecular weight HA (1%) and ADM (2%) in osteoarthritis [33-35].

The aim of the present study was twofold. First, to gain new information on the antioxidant effect of ADM and its ability to protect HA against oxidative depolymerization. Second, to report our clinical experience on two patients with primary and secondary AC respectively, managed through a multimodal approach including tailored physical therapy and intra-articular injections of HA and ADM.

Material and Methods

In vitro Chemical Study

Materials

Sodium hyaluronate and Adelmidrol solution of the same standard quality as the commercially available formulation (Hyadrol®, factory code 6301) was kindly donated by Epitech Group S.p.A., Milan, Italy. Hyadrol® is a steam sterilized solution for intra-articular use in the form of ready-to-administer prefilled syringes and contains high molecular weight HA (1%, molecular weight 1300-2000 kDa), ADM (2%), sodium chloride, potassium dihydrogen phosphate, sodium hydrate, EDTA disodium and water. A similar solution was prepared with the exception of ADM, and thereafter referred to as solution A. Sodium chloride, potassium dihydrogen phosphate, sodium hydrate and EDTA were supplied by ACEF S.p.A. (Fiorenzuola d'Arda, Piacenza, Italy). Copper sulphate and hydrogen peroxide (30%) were purchased from Merck KGaA (Darmstadt, Germany). High molecular weight sodium hyaluronate (molecular weight 1500 kDa, batch # PH14202C) obtained from bacterial fermentation was provided by HTL Biotechnology (Javene, France).

Hyaluronan Oxidative Degradation

Three aliquots of solution A (100 mL each) were collected (i.e., A1, A2 and A3). A1 was conserved as such (control solution). A volume of 170 µL $CuSO_4$ (0.1M) and 25 µL of EDTA (0.22 mM) was added to A2 and A3, stirred for 30 s and incubated at 37°C for 24 h, as previously described [36]. ADM (2 g) was then added to solution A2, in order to achieve the final 2% concentration of Hyadrol®. A volume of 1 mL H_2O_2 (30%) was added to both A2 and A3, followed by 30-s stirring. In this condition, hydrogen peroxide (H_2O_2) reacts with cupric ions (Cu^{2+}) generating hydroxyl

radicals responsible of HA oxidative degradation [36].

Rotational Viscometry

Degradation of HA was assessed by the changes of the dynamic viscosity [37]. A rotational viscometer was used (Brookfield RVDV-II+P, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Dynamic viscosity of the sample solutions was measured at 37°C for up to 4h and expressed in centipoise (cP), with one cP being one millipascal-second (mPa·s). The viscometer was equipped with spindle N. 3, rotation speed 50 rpm.

In vitro Cell Study

Culturing and Treatment

The monocyte/macrophage J774-A1 cells ($8x10^5$ cells) were plated into 24 well tissue culture plates and grown to confluence (48h at 37°C). Subsequently, cells were washed twice in 1X phosphate buffered saline (PBS) and cultured in F-12/FCS 1% for the period of the experiment. After 6h pre-treatment with Adelmidrol (0, 10, 50, 100 µM) cells were stimulated with hydrogen peroxide (H_2O_2, 200 µM) for 10 min. At the end of each experiment, the assays described below were performed.

Cell Protection against Oxidative Damage

To assess cells viability, cells were incubated at 37°C with 0.2 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) for 1 h and the resulting solution was measured for optical density (OD) at 550 nm (OD550) using a microplate reader, as previously described [38].

Measurement of Nitrite levels

Total nitrite level, as an indicator of nitric oxide (NO) synthesis, was measured in the supernatant. Briefly, nitrate in the medium was reduced to nitrite by incubation with nitrate reductase (670 mU/mL) and β-nicotinamide adenine dinucleotide 3-phosphate (160 mM) at room temperature for 3 h. Total nitrite concentration was then measured using the Griess reaction by adding 100 µl Griess reagent (0.1% (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride in H_2O and 1% (w/v) sulfanilamide in 5% (v/v) concentrated H_3PO_4; volume 1:1) to the 100 µl sample. OD550 was measured using an enzyme-linked immunosorbent assay microplate reader (Tecan, Männedorf, Switzerland). Nitrite concentrations were calculated by comparison with OD550 of standard solutions of sodium nitrite prepared in H_2O, as previously described [39].

Determination of intracellular ROS

A chemically reduced, acetylated form of fluorescein (i.e., carboxy-H2DCFDA dye) was used as an indicator for reactive oxygen species (ROS) in cells, as previously described [40,41]. Briefly, cells were trypsinized after treatment and then washed twice with 1x washing buffer. Cells were then incubated with carboxy-H2DCFDA (10 µM final concentration) at 37°C in the dark for 30 min. The fluorescence microplate reader detected the light emission.

Statistical analysis

All values from the *in vitro* cell study are expressed as mean ± standard error of the mean (SEM). The results were analyzed by one-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test for multiple comparisons. A P-value of less than 0.05 was considered significant.

Case Presentation

Case 1

A 57-year-old woman visited the Functional Recovery and Rehabilitation Unit at the Belluno Hospital because of pain worsening at the left shoulder. Pain was long-lasting (more than 3 months) and intermittent at rest and at night, severely interfering with sleep. Supraspinatus tendinopathy was suspected on musculoskeletal ultrasound and confirmed with magnetic resonance (Figure 1).

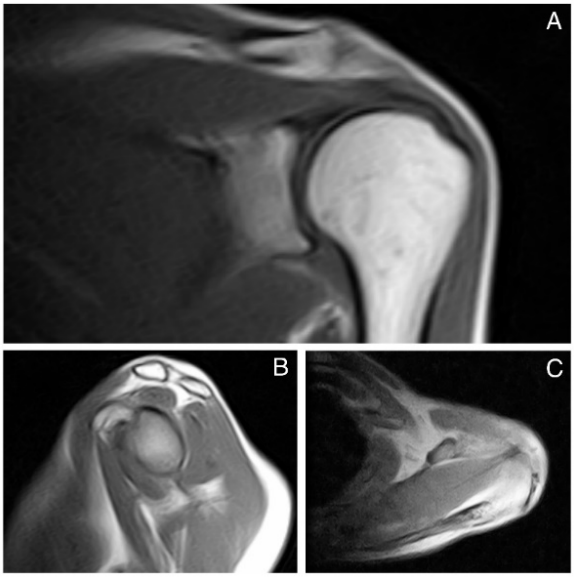

Figure 1: MR of the left shoulder. Supraspinatus tendinopathy, with tendon alteration and thickening, in sagittal (A), frontal (B) and coronal projections (C) is evident.

No further abnormalities were displayed, not even on X-ray investigation. At clinical evaluation, the patient presented with severe stiffness of the shoulder, internal rotation limited to 10-15

degrees, no external rotation and severe pain at shoulder movement in any direction, i.e., 9 out of 10 on the Numerical Rating Scale (NRS). Abnormal scapular motion with moderate dyskinesia was also evident. Diagnosis of primary adhesive capsulitis with supraspinatus tendinopathy was made. For antalgic purposes, a single intra-articular infiltration with triamcinolone hexacetonide (40 mg\mL) and lidocaine (20 mg\mL) was performed. A prescription for an initial 10-session physical therapy (PT) was issued, in order to reduce pain and preserve shoulder mobility and function. The patient attended a 40-minute PT session every other day, consisting of active assisted mobilization and passive gentle traction of the left shoulder ("pompage"). Active assisted mobilization was performed during the acute phase to promote soft tissue stretching and joint lubrication (by stimulating hyaluronic acid production), as well as to restore proprioception and ROM. Passive PT was carried out through progressive mobilization techniques tailored to the patient's response. Low-intensity continued ultrasound (LICUS) was also used for both pain and stiffness relieving purposes (41). In each PT session, LICUS lasted 12 minutes at 1 MHz, 1.5-1.7 W/cm^2 intensity range (HC SOUND, Elettronica Pagani, Paderno Dugnano, Milan, Italy).

Case 2

A 71-year-old woman was referred to the Functional Recovery and Rehabilitation Unit of the Belluno Hospital because of complications after a proximal humerus fracture (Figure 2A).

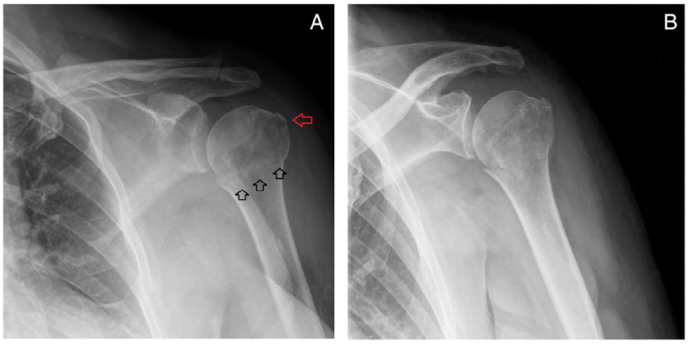

Figure 2: Orthogonal radiographs of the left shoulder - (A) Acute proximal humeral fractures at the surgical neck (black arrows) and greater tuberosity level (red arrow). (B) Evidence of initial consolidation after 30-day shoulder brace immobilization.

The fracture was caused by a fall from standing height and was conservatively managed with a shoulder brace for 30 days. Thereafter, an acceptable consolidation was seen at radiographic examination (Figure 2B) and the patient was referred to our Rehabilitation Unit. At the first rehabilitation visit, the patient held her left arm in marked adduction and internal rotation. An extensive hematoma in the left upper limb was also present. Pain at NRS was scored 9 and ROM was very limited in either abduction, internal rotation, external rotation, or flexion. Post-traumatic / post-immobilization shoulder stiffness was initially diagnosed. A 10-session PT on an every-other-day regimen was recommended. Each session lasted 40 minutes and consisted of passive PT and magnetic field therapy. Passive PT was performed through relaxation and alignment techniques to preserve the patient's psychophysical well-being and correct poor posture responsible for functional limitations. Passive mobilization to increase joint mobility was also performed. Moreover, 25-minute magnetic field therapy per session was delivered at 30 Gauss and 100 Hz (MT- Chinesport Italia, Udine - Italy) with the aim of speeding up consolidation.

Treatment Monitoring

For both patients, treatment effect was monitored at each control visit by measuring:

(i) pain severity, assessed by an 11-point scale (0 meaning no pain and 10 meaning the worst pain imaginable), i.e., the Numeric Rating Scale (NRS) [42]; (ii) upper limb function, assessed through the Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire, ranging from 0 (no disability) to 100 (most severe disability) [43]. DASH measures impairment, activity limitations and participation restriction [44], allowing the patient to rate difficulty and interference with activities of daily living (ADL).

Results

Adelmidrol Protects Hyaluronan against Oxidative Degradation

The kinetics of the hyaluronan oxidative degradation was monitored by changing the dynamic viscosity of the solution. The experimental oxidative system ($CuSO_4$ + H_2O_2) effectively induced HA degradation as shown by the fast decrease of dynamic viscosity in solution A3 (Figure 3, red curve). As evident from the blue curve in Figure 3, the oxidative depolymerization of HA was greatly counteracted by ADM. At 30 min, more than 50% inhibition of oxidative degradation was observed.

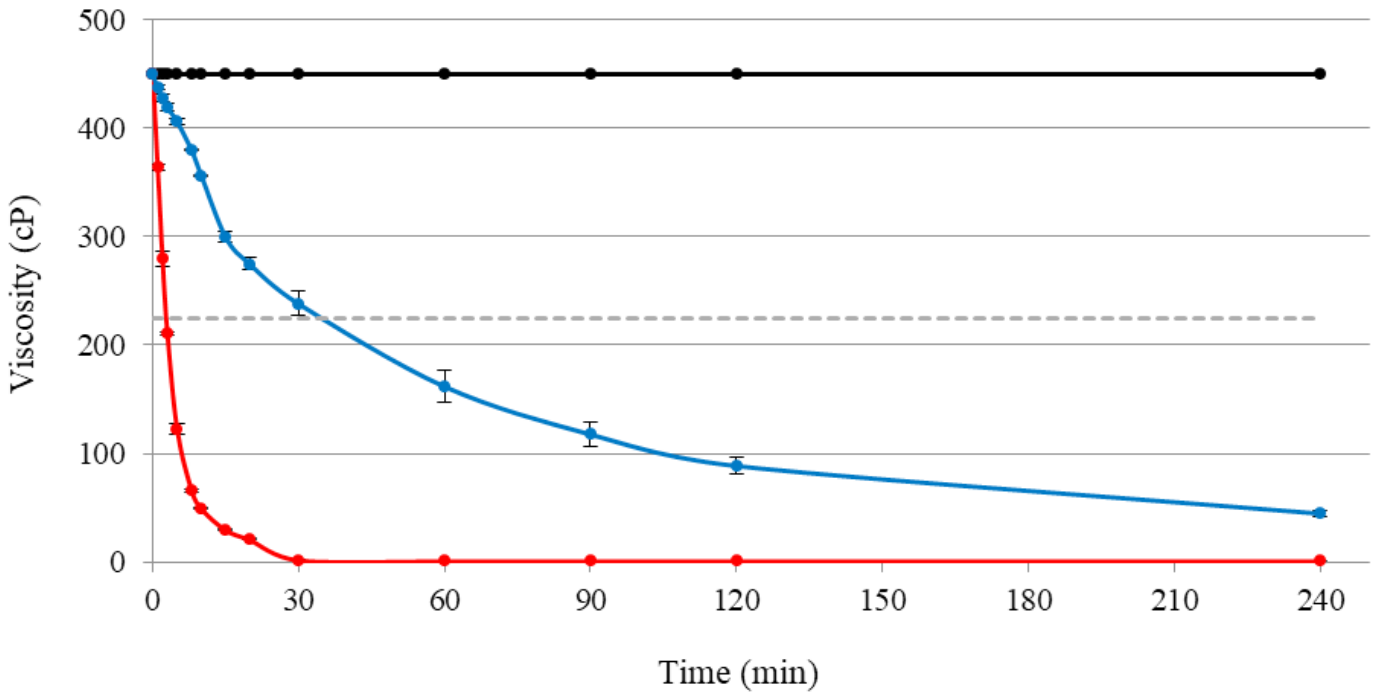

Figure 3: Time-dependent changes in dynamic viscosity of HA solutions exposed to oxidative degradation initiated by cupric ions (Cu^{2+}) and hydrogen peroxide (H_2O_2). In 30 min, viscosity dropped from 450 to 0 cP without the addition of ADM (red curve, A3 solution), while it was higher than 50% of the original value with the addition of ADM (blue curve, A2 solution). Grey dotted line represents 50% viscosity. Black curve represents the control HA solution, unexposed to oxidative degradation (A1 solution). Data represent the mean ± SEM of three independent experiments. See Materials and Methods for further details.

Adelmidrol protects macrophages against oxidative damage and decreases free radical production

As shown in Figure 4A, pre-treatment with ADM showed a dose-dependent protective effect against H_2O_2 oxidative stress, preserving cell viability. Notably, the lowest concentration (10 μM) was significantly effective and was used in the following experiments. Cell stimulation with H_2O_2 increased the levels of NO_2^-, with the effect being significantly counteracted by ADM (Figure 4B). Similar results were observed for the intracellular ROS production (Figure 4C).

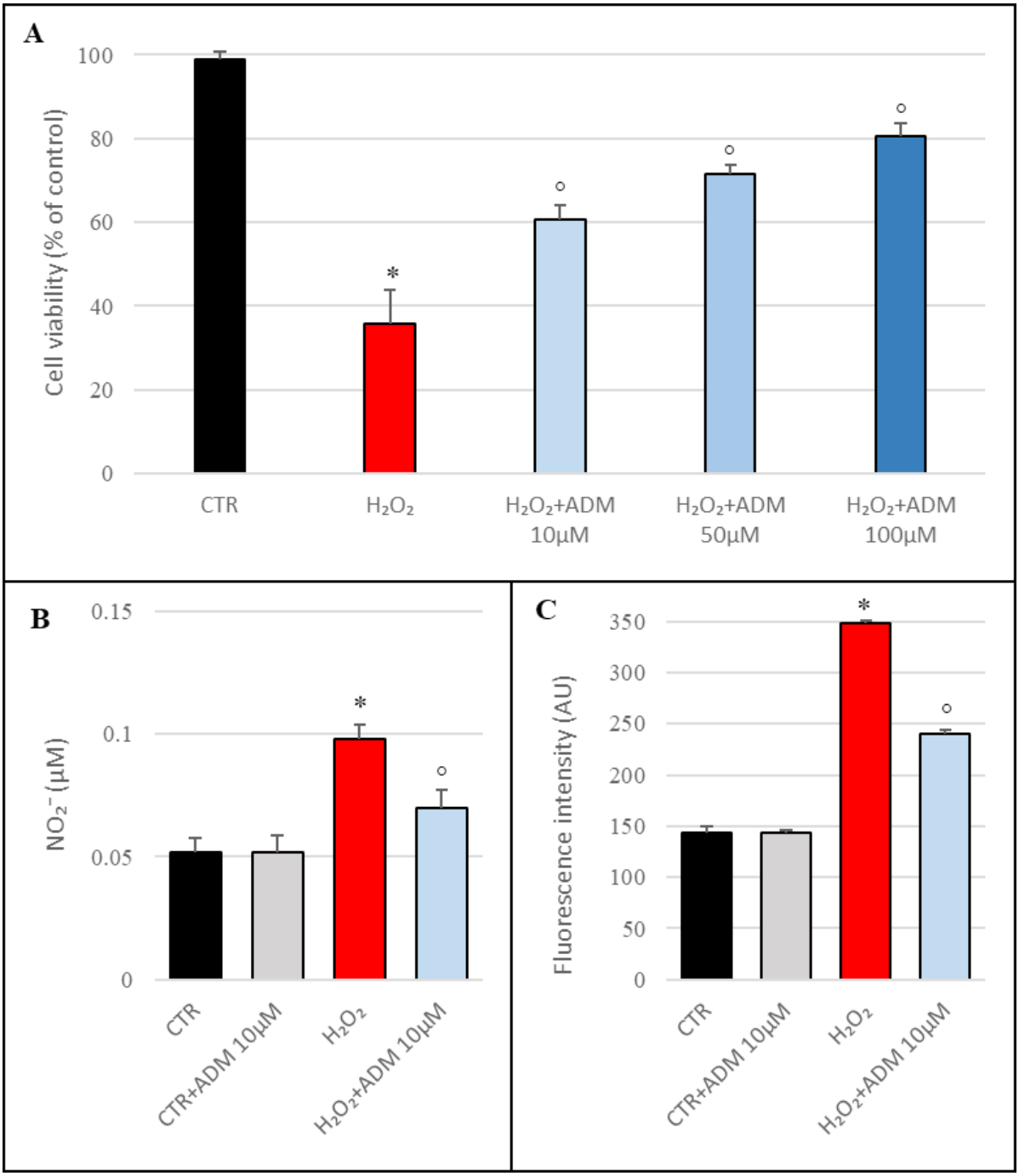

Figure 4: Antioxidant activity of Adelmidrol on H_2O_2-stimulated monocyte/macrophage J774-A1 cells. The antioxidant activity was measured as (A) cells protection against H_2O_2-induced cell death, (B) nitrite release and (C) intracellular ROS production. The lowest dose of Adelmidrol that was effective in counteracting H_2O_2-induced cell death (i.e., 10 µM) was used in the nitrite (B) and ROS assays (C). * $P < 0.001$ vs CTR; ° $P < 0.05$ vs H_2O_2. CRT = control; ADM = Adelmidrol.

Case Report

Case 1

At the first check-up (i.e., after 4-week PT), a slight improvement in night pain was reported (NRS 8). Stiffness and pain upon active and passive movements were still severe. The DASH score was 67.5. A further 10-session PT (same type, duration, and frequency as the previous one) was planned. Briefly, manual capsule stretching, antalgic maneuvers and massage to reduce muscle contractures were initially performed. Next, hold-relax and contract-relax proprioceptive neuromuscular facilitation techniques were performed to regain shoulder muscle tone and proprioception. In order to synergize with PT effect, high molecular weight HA (1%) protected by ADM (2%) against oxidative degradation (Hyadrol®, 2 mL) was injected and two more injections were planned fortnightly. At the second check-up, two weeks after the third intra-articular injection, the patient showed a marked improvement in pain (NRS 1) and motor function, with a total recovery of the physiological ROM. The rotator cuff specific tests (i.e., lift-off, Napoleon, painful arc, Yocum, and Jobe's tests) returned negative results. Scapular dyskinesia was no longer present. The DASH questionnaire revealed a significant improvement in ADL (score 2.5). At the telephone check-up, 6 months after the last visit, the patient reported complete motor relief, with only minimal pain (NRS 3). Pain changes are summarized in Figure 5.

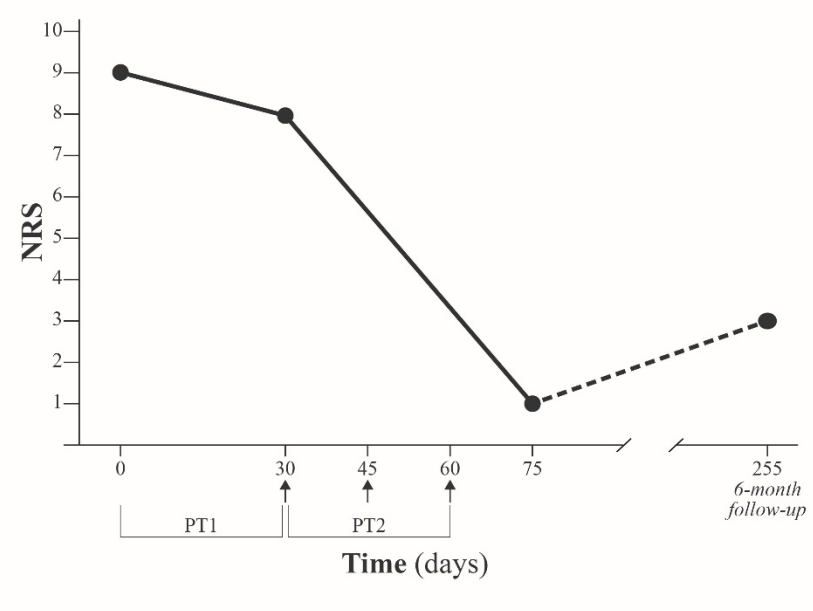

Figure 5: Change in pain severity during and after (6-month follow-up) the combined PT and intra-articular management of case 1. Arrows indicate when intra-articular HA+ADM injections were performed.

Case 2

After the first PT regimen, the patient still scored pain as quite severe (NRS 7). Ten more sessions of active-assisted PT (40 minutes each) were planned, with active exercises combined with magnetic field therapy (same regimen as the initial protocol) in order to improve consolidation. During each PT session, antalgic maneuvers, techniques to reduce muscle contractures and active exercises were performed for improving shoulder muscle tone and proprioception. At the end of the second PT rehabilitation phase, left shoulder mobility and ROM were improved, albeit the patient still complained severe stiffness and pain on rotation and overhead motion (NRS 6). The DASH questionnaire scored 15.8. A diagnosis of secondary AC was then made. An intra-articular course of three fortnightly injections with high molecular weight HA 1% and 2% ADM (Hyadrol®, 2 mL) was initiated in combination with a third 10-session active-assisted PT. Each 40-minute session consisted in capsule stretching, mobilization techniques and, in the last phase, active exercises. The aim was multifaceted: to strengthen the rotator cuff, promote the recovery of ROM, drain periarticular oedema, facilitate venous return, and prevent intra-articular adhesions. Magnetic field therapy was no longer performed, since consolidation was considered complete (no pain or motion at the fracture site). At the last check-up (15 days after the third and last intra-articular injection), the patient achieved

a total recovery of active ROM, and an improvement in ADL (DASH score 2.5), in the absence of pain (NRS 0). At the telephone check-up, 6 months later, the patient reported persistence of the acquired relief on movements and remained pain free (NRS 0). Pain changes along time (i.e., during rehabilitation management and after 6-month follow-up) are summarized in Figure 6.

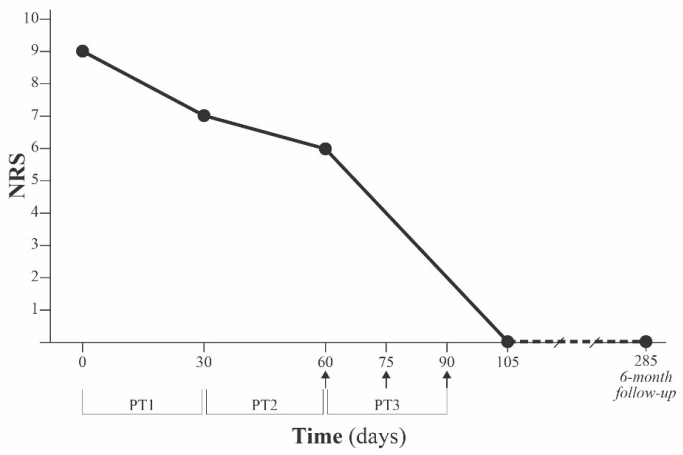

Figure 6: Change in pain severity during and after (6-month follow-up) the combined rehabilitation and intra-articular protocol used in case 2. Arrows indicate when intra-articular HA+ADM injections were performed.

Discussion

Although AC is considered a self-limited disease, symptoms are usually very disabling and may persist for years [2,19]. Therapies for AC patients can be divided into symptomatic treatments (focused on pain management) and capsulitis-modifying interventions (aimed to restore the shoulder to a functional and painless joint) [45]. Pharmacological therapy, mainly symptomatic in nature, relays on oral (i.e., non-steroidal anti-inflammatory drugs and corticosteroids) or intra-articular steroid injections. Both interventions generally allow for short-term pain relief [45]. Additionally, short-term relief can also be obtained following intra-articular HA injections in AC patients [46]. Physical therapy is a mainstay in the AC-modifying interventions and is almost universally recommended [47]. Nonetheless, there is limited evidence to support the use of PT as a single measure and it is often combined with other treatment modalities [47]. Here we report the successful long-term management of two cases of AC with different presentations. Interventions included multiple and tailored PT programs combined with three fortnightly intra-articular injections of a new oxidant-protected formulation of HA, containing ADM. ADM had emerged as a "general" antioxidant from previous studies [30-32] but its protective effect against oxidative degradation of HA had not been addressed yet.

In a simple *in vitro* assay, we have shown for the first time that the addition of 2% ADM to 1% high molecular weight HA solution inhibits free radical-induced degradation of HA, as properly monitored by changes in dynamic viscosity [48]. Decrease of HA viscosity is, in fact, known to reflect HA degradation [49]. In particular, ADM behaved as a preventive antioxidant, as it was loaded into the HA solution before hydrogen peroxide was added. One may envision that ADM eventually induced a conformational change in the HA molecule resulting in increased protection against oxidative degradation. Alternatively, the decrease in the HA viscosity drop was probably due to the ADM ability to scavenge hydroxyl radicals generated following H_2O_2 addition, similar to thiol compounds [37]. The hypothesis is in line with the known scavenging activity of azelaic acid [50], which might be retained once azelaic acid is amide-linked to ethanolamine, as in the ADM molecule. Actually, the hydroxyl radical scavenging activity of ADM was also supported by the second set of *in vitro* experiment we performed on a macrophage cell line.

Macrophages are one of the most active cell type in free radical generation [51]. Here we have shown that ADM dose-dependently increases cell survival against hydrogen peroxide-induced oxidative stress, and concurrently counteracts the macrophage generation of reactive oxygen and nitrogen species. Again, the scavenging of hydroxyl radicals spontaneously generated from hydrogen peroxide is one of the most plausible explanations for the antioxidant effect of ADM observed in macrophages. It is well known that the therapeutic effect of intra-articular hyaluronic acid injections directly depends on the molecular weight of the biopolymer [52]. The antioxidant properties of ADM specifically addressed to maintain HA molecular weight (and rheological properties accordingly) may thus represent a unique advantage in the management of conditions where oxidative stress plays a pathogenetic role, like AC [10]. This is the reason why we decided to investigate the newly available intra-articular formulation of high molecular weight HA and ADM in the management of two patients with AC partially responding to PT. The clinical presentation of the two cases was very different. In the first one, the diagnosis of primary AC with supraspinatus tendinopathy was readily made and the corresponding multimodal treatment program was addressed to pain relief and functional rehabilitation. A single intra-articular steroid injection and two phases of active and passive shoulder mobilization together with LICUS for an overall 2-month duration were performed. The addition of three fortnightly intra-articular HA+ADM injections during the second PT phase markedly improved the shoulder mobility and relieved pain. In the second case, the AC diagnosis took longer and treatment was originally addressed to post-traumatic consolidation and joint stiffness. Passive manipulations and magnetic field therapy on an every-other-day regimen were performed for two months and

only after this period a diagnosis of AC was clear. Although the intra-articular injections with HA+ADM were postponed in the second patient compared to first one, as they began two months after clinical presentation, benefits on shoulder mobility, ADL and perceived pain were clearly seen. The most intriguing aspect of the presented cases is the finding at long-term follow-up. In both patients, in fact, complete pain relief was obtained a couple of weeks after the last intra-articular injection of oxidant-protected HA, with the effect being maintained six months later. This is a very positive outcome based on our clinical experience. Usually, PT takes longer to get clinical benefit and the effect of intra-articular HA injections is generally shorter, being maintained on average up to three months after the treatment end [53].

Conclusions

The present study suggests that the duration of the effect of intra-articular HA injections may be prolonged in AC patients if the lubricating biopolymer is preserved by oxidative degradation thanks to ADM. The positive and long-term outcome of the two AC patients here presented supports the hypothesis that the new intra-articular formulation of high molecular weight HA 1% and ADM 2% may provide an advantage over HA used singly. Further well-designed clinical studies are warranted to confirm the clinical benefit of this newly available oxidant-protected HA in AC and other joint disorders.

Conflict of Interest

GM and FG are consultant for and employed by Epitech Group S.p.A., respectively. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Manske RC, Prohaska D (2008) Diagnosis and Management of Adhesive Capsulitis. Curr Rev Musculoskelet Med 1(3-4): 180-189.
2. St Angelo JM, Taqi M, Fabiano SE (2024) Adhesive Capsulitis. In Stat Pearls, Stat Pearls Publishing, Treasure Island.
3. Dias R, Cutts S, Massoud S (2005) Frozen Shoulder. BMJ 331: 1453-1456.
4. Farinelli L, Aquili A, Mattioli-Belmonte M, Manzotti S, D'Angelo, et al. (2022) Synovial Mast Cells from Knee and Hip Osteoarthritis: Histological Study and Clinical Correlations. J Exp Orthop 9(1): 13.
5. Liu H, Yu B, Deng Z, Zhao H, Zeng A, et al. (2023) Role of Immune Cell Infiltration and Small Molecule Drugs in Adhesive Capsulitis: Novel Exploration Based on Bioinformatics Analyses. Front Immunol 9:14: 1075395.
6. Hand GCR, Athanasou NA, Matthews T, Carr AJ (2007) The Pathology of Frozen Shoulder. J Bone Joint Surg Br 89(7): 928-932.
7. Turan S, Deveci A, Kulaçoğlu S, Bingöl O, Özdemir G (2020) There Is a Correlation between Histopathological Findings of Joint Capsule and Synovium, and Postoperative Clinical Outcomes and Treatment in Patients with Isolated Type II Superior Labrum Anterior Posterior Lesions. Jt Dis Relat Surg 3: 440-448.
8. Usher KM, Zhu S, Mavropalias G, Carrino JA, Zhao J, et al. (2019) Pathological Mechanisms and Therapeutic Outlooks for Arthrofibrosis. Bone Res 7: 9.
9. Lewis JS, Sandford FM (2009) Rotator Cuff Tendinopathy: Is There a Role for Polyunsaturated Fatty Acids and Antioxidants? J Hand Ther 22: 49-55.
10. Kil KM, Jung SH, Jeon IS, Cho BK, Park JK, (2017) Reactive Oxygen Species (ROS) Induces Distinct Gene Expression in Human Fibroblast like Synoviocytes (HFLS). Journal of Biomedical and Translational Research 18: 130-135.
11. Pigman W, Rizvi S, Holley HL (1961) Depolymerization of Hyaluronic Acid by the ORD Reaction. Arthritis Rheum 4: 240-252.
12. Soltés L, Mendichi R, Kogan G, Schiller J, Stankovska M, et al. (2006) Degradative Action of Reactive Oxygen Species on Hyaluronan. Biomacromolecules 7: 659-668.
13. Duan J, Kasper DL (2011) Oxidative Depolymerization of Polysaccharides by Reactive Oxygen/Nitrogen Species. Glycobiology 21: 401-409.
14. Kosinska MK, Ludwig TE, Liebisch G, Zhang R, Siebert HC, et al. (2015) Articular Joint Lubricants during Osteoarthritis and Rheumatoid Arthritis Display Altered Levels and Molecular Species. PLoS One 10: e0125192.
15. Yoshida M, Sai S, Marumo K, Tanaka T, Itano N, et al. (2004) Expression Analysis of Three Isoforms of Hyaluronan Synthase and Hyaluronidase in the Synovium of Knees in Osteoarthritis and Rheumatoid Arthritis by Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction. Arthritis Res Ther 6: R514-520.
16. Dahl LB, Dahl IM, Engström-Laurent A, Granath K (1985) Concentration and Molecular Weight of Sodium Hyaluronate in Synovial Fluid from Patients with Rheumatoid Arthritis and Other Arthropathies. Ann Rheum Dis 44(12): 817-822.
17. Lee LC, Lieu FK, Lee HL, Tung TH (2015) Effectiveness of Hyaluronic Acid Administration in Treating Adhesive Capsulitis of the Shoulder: A Systematic Review of Randomized Controlled Trials. Biomed Res Int 314120.
18. Mao B, Peng R, Zhang Z, Zhang K, Li J, et al. (2022) The Effect of Intra-Articular Injection of Hyaluronic Acid in Frozen Shoulder: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Orthop Surg Res 17: 128.
19. Pietrzak M (2016) Adhesive Capsulitis: An Age-Related Symptom of Metabolic Syndrome and Chronic Low-Grade Inflammation? Med Hypotheses 88: 12-17.

20. Song A, Higgins LD, Newman J, Jain NB (2014) Glenohumeral Corticosteroid Injections in Adhesive Capsulitis: A Systematic Search and Review. PM R 6: 1143-1156.

21. Drafi F, Bauerova K, Valachova K, Ponist S, Mihalova D, et al. (2010) Carnosine Inhibits Degradation of Hyaluronan Induced by Free Radical Processes *in Vitro* and Improves the Redox Imbalance in Adjuvant Arthritis *In Vivo*. Neuro Endocrinol Lett 31 Suppl 2, 96-100.

22. Impellizzeri D, Di Paola R, Cordaro M, Gugliandolo E, Casili G, et al, (2016) Adelmidrol, a Palmitoylethanolamide Analogue, as a New Pharmacological Treatment for the Management of Acute and Chronic Inflammation. Biochem Pharmacol 119: 27-41.

23. Levi-Montalcini R, Skaper SD, Dal Toso R, Petrelli L, Leon A (1996) Nerve Growth Factor: From Neurotrophin to Neurokine. Trends Neurosci 19: 514-520.

24. Skaper SD, Facci L, Giusti P (2013) Glia and Mast Cells as Targets for Palmitoylethanolamide, an Anti-Inflammatory and Neuroprotective Lipid Mediator. Mol Neurobiol 48: 340-352.

25. Roviezzo F, Rossi A, Caiazzo E, Orlando P, Riemma MA, et al. (2017) Palmitoylethanolamide Supplementation during Sensitization Prevents Airway Allergic Symptoms in the Mouse. Front Pharmacol 8: 857.

26. Petrosino S, Schiano Moriello A, Verde R, Allarà M, Imperatore R, et al. Palmitoylethanolamide Counteracts Substance P-Induced Mast Cell Activation *in Vitro* by Stimulating Diacylglycerol Lipase Activity. J Neuroinflammation 16: 274.

27. Micheli L, Lucarini E, Nobili S, Bartolucci G, Pallecchi M, et al. (2024) Ultramicronized N-Palmitoylethanolamine Contributes to Morphine Efficacy Against Neuropathic Pain: Implication of Mast Cells and Glia. Curr Neuropharmacol 22: 88-106.

28. Petrosino S, Puigdemont A, Della Valle MF, Fusco M, Verde R, et al. (2016) Adelmidrol Increases the Endogenous Concentrations of Palmitoylethanolamide in Canine Keratinocytes and Down-Regulates an Inflammatory Reaction in an *in Vitro* Model of Contact Allergic Dermatitis. Vet J 207: 85-91.

29. Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, et al. (2008) Characterisation of the Cannabinoid Receptor System in Synovial Tissue and Fluid in Patients with Osteoarthritis and Rheumatoid Arthritis. Arthritis Res Ther 10: R43.

30. Palenca I, Seguella L, Zilli A, Basili Franzin S, Del Re A, et al. (2023) Intrarectal Administration of Adelmidrol plus Hyaluronic Acid Gel Ameliorates Experimental Colitis in Mice and Inhibits Pro-Inflammatory Response in Ex Vivo Cultured Biopsies Derived from Ulcerative Colitis-Affected Patients. Int J Mol Sci 25: 165.

31. Wu M, Liu X, Yu Q, Shi J, Guo W, et al. (2024) Adelmidrol Ameliorates Liver Ischemia-Reperfusion Injury through Activating Nrf2 Signaling Pathway. Eur J Pharmacol 964: 176224.

32. Fusco R, Cordaro M, Genovese T, Impellizzeri D, Siracusa R, et al. (2020) Adelmidrol: A New Promising Antioxidant and Anti-Inflammatory Therapeutic Tool in Pulmonary Fibrosis. Antioxidants (Basel) 9: 601.

33. Di Paola R, Fusco R, Impellizzeri D, Cordaro M, Britti D, et al. (2016) Adelmidrol, in Combination with Hyaluronic Acid, Displays Increased Anti-Inflammatory and Analgesic Effects against Monosodium Iodoacetate-Induced Osteoarthritis in Rats. Arthritis Res Ther 18: 291.

34. Guida F, Rocco M, Luongo L, Persiani P, Vulpiani MC, et al. (2022) Targeting Neuroinflammation in Osteoarthritis with Intra-Articular Adelmidrol. Biomolecules 12: 1453.

35. Vulpiani MC, Vetrano M, Nusca SM, Latini E, Santoboni F, et al. (2023) Hyaluronic Acid Alone versus Hyaluronic Acid Associated with Adelmidrol for Intra-Articular Treatment of Knee Osteoarthritis: A Long-Term Follow-Up. Int J Physiother Res 11: 4453-4460.

36. Mendoza G, Alvarez AI, Pulido MM, Molina AJ, Merino G, et al. (2007) Inhibitory Effects of Different Antioxidants on Hyaluronan Depolymerization. Carbohydr Res 342: 96-102.

37. Valachová K, Vargová A, Rapta P, Hrabárová E, Dráfi F, et al. (2011 Aurothiomalate as Preventive and Chain-Breaking Antioxidant in Radical Degradation of High-Molar-Mass Hyaluronan. Chem Biodivers 8: 1274-1283.

38. Paterniti I, Impellizzeri D, Cordaro M, Siracusa R, Bisignano C, et al. (2017) The Anti-Inflammatory and Antioxidant Potential of Pistachios (Pistacia Vera L.) *In Vitro* and *In Vivo*. Nutrients 9: 915.

39. Paterniti I, Cordaro M, Campolo M, Siracusa R, Cornelius C, et al. (2014) Neuroprotection by Association of Palmitoylethanolamide with Luteolin in Experimental Alzheimer's Disease Models: The Control of Neuroinflammation. CNS Neurol Disord Drug Targets 13: 1530-1541.

40. Perez AP, Casasco A, Schilrreff P, Tesoriero MVD, Duempelmann L, et al. (2014) Enhanced Photodynamic Leishmanicidal Activity of Hydrophobic Zinc Phthalocyanine within Archaeolipids Containing Liposomes. Int J Nanomedicine 9: 3335-3345.

41. Riolo K, Rotondo A, La Torre GL, Marino Y, Franco GA, et al. (2023) Cytoprotective and Antioxidant Effects of Hydrolysates from Black Soldier Fly (Hermetia Illucens). Antioxidants (Basel) 12: 519.

42. Haefeli M, Elfering A (2006) Pain Assessment. Eur Spine J 15 Suppl 1, S17-24.

43. Padua R, Padua L, Ceccarelli E, Romanini E, Zanoli G, et al. (2003) Italian Version of the Disability of the Arm, Shoulder and Hand (DASH) Questionnaire. Cross-Cultural Adaptation and Validation. J Hand Surg Br 28: 179-186.

44. Dixon D, Johnston M, McQueen M, Court-Brown C (2008) The Disabilities of the Arm, Shoulder and Hand Questionnaire (DASH) Can Measure the Impairment, Activity Limitations and Participation Restriction Constructs from the International Classification of Functioning, Disability and Health (ICF). BMC Musculoskelet Disord 9: 114.

45. Le HV, Lee SJ, Nazarian A, Rodriguez EK (2017) Adhesive Capsulitis of the Shoulder: Review of Pathophysiology and Current Clinical Treatments. Shoulder Elbow 9: 75-84.

46. Harris JD, Griesser MJ, Copelan A, Jones GL (2011) Treatment of Adhesive Capsulitis with Intra-Articular Hyaluronate: A Systematic Review. Int J Shoulder Surg 5: 31-37.

47. Patel R, Urits I, Wolf J, Murthy A, Cornett EM, et al. (2020) A Comprehensive Update of Adhesive Capsulitis and Minimally Invasive Treatment Options. Psychopharmacol Bull 50(4 Suppl 1): 91-107.

48. Valachová K, Šoltés L (2021) Assessment of the Substance Antioxidative Profile by Hyaluronan, Cu(II) and Ascorbate. Pharmaceutics 13: 1815.

49. Banasova M, Sasinkova V, Mendichi R, Perecko T, Valachova K, (2012) Juranek, I, Soltes, L. Free-Radical Degradation of High-Molar-Mass Hyaluronan Induced by Weissberger's Oxidative System: Potential Antioxidative Effect of Bucillamine. Neuro Endocrinol Lett 33 Suppl 3: 151-154.

50. Passi S, Picardo M, De Luca C, Breathnach AS, Nazzaro-Porro M (1991) Scavenging Activity of Azelaic Acid on Hydroxyl Radicals "*In Vitro*." Free Radic Res Commun 11: 329-338.

51. Canton M, Sánchez-Rodríguez R, Spera I, Venegas FC, Favia M, et al. (2021) Reactive Oxygen Species in Macrophages: Sources and Targets. Front Immunol 12: 734229.

52. Snetkov P, Zakharova K, Morozkina S, Olekhnovich R, Uspenskaya M (2020) Hyaluronic Acid: The Influence of Molecular Weight on Structural, Physical, Physico-Chemical, and Degradable Properties of Biopolymer. Polymers (Basel) 12: 1800.

53. Di Giacomo G, de Gasperis N (2017) Hyaluronic Acid Intra-Articular Injections in Patients Affected by Moderate to Severe Glenohumeral Osteoarthritis: A Prospective Randomized Study. Joints 5: 138-142.