A PILOT STUDY TO COMPARE TWO DIFFERENT HYALURONIC ACID COMPOUNDS FOR TREATMENT OF KNEE OSTEOARTHRITIS

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Osteoarthritis is characterized by progressive articular cartilage degeneration, changes in subchondral bone and synovial inflammation, leading to pain and disability. Viscosupplementation with hyaluronic acid has been widely investigated due to the viscoelastic properties of this compound to manage pain improving the ability to perform daily activities in patients affected by osteoarthritis. In the present study we investigated the clinical effectiveness of viscosupplementation with a new highly cross-linked hyaluronic acid, Variofill®, in patients affected by bilateral knee osteoarthritis in comparison with the widely used Synvisc®. A total of 20 patients, aged between 24-74 years and affected by bilateral knee osteoarthritis, participated in this pilot randomized triple-blind clinical study. They received two injections (2 ml each) of Synvisc® in their left knee and 2 injections (2 ml each) of Variofill® in their right knee spaced 15 days apart. Visual Analogue Scale and Western Ontario McMaster Universities Osteoarthritis Index score were used to evaluate the efficacy of hyaluronic acid injections before and 3 and 6 months after treatment. Both treatment regimens resulted in a significant improvement *vs* baseline in all endpoints at 3 and 6 months ($p < 0.001$). Treatment with Variofill® resulted in a high percentage improvement in Visual Analogue Scale pain, Western Ontario McMaster Universities Osteoarthritis Index score pain and physical activity, when compared to Synvisc® viscosupplementation, at 6 months ($p < 0.05$). These results are encouraging for larger clinical trials with Variofill® in larger cohorts of patients affected by osteoarthritis of the knee.

Osteoarthritis (OA) represents the most common form of arthritis affecting the aging population on a worldwide scale, leading to pain and disability (1). OA has a high impact on the lower limbs, namely the knee and hip, causing focal cartilage breakdown, osteophytes, subchondral sclerosis, diffuse collagen thickness, and synovial reaction that can lead to frequent synovial fluid effusion (2, 3). Patient's education for prevention, including weight reduction and physical exercise, is highly recommended (2, 4). Treatment is often based on drugs including glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) which, however, do not provide a complete improvement and are associated with side effects (4). Further therapy, including anti-cytokine therapy, gene therapy, delivery of growth factors, stem-cell therapy, and new lubricant agents, such as lubricin, has been more recently proposed (5).

Key words: osteoarthritis, hyaluronic acid, viscosupplementation, Synvisc®, Variofill®

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The Osteoarthritis Research Society International (OARSI) has warned about self-limiting anti-inflammatory drug consumption, advising to maintain a minimum effective dosage for the shortest time possible, due to the numerous untoward effects, including gastrointestinal haemorrhage, which positively correlates with the patients' age, and to avoid long-term use (6-8). The healthy knee joint surface is lubricated by 1-2 ml of synovial fluid, which is not only a simple biofluid, but also a viscoelastic mix of glycosaminoglycan, namely hyaluronic acid (HA) and proteins secreted by the synovial lining cells (1). HA function is essential for the maintenance of articular homeostasis since it exerts a key function as a shock absorber, and lubricates the cartilage in order to avoid friction and early breakdown due to lysosomal activity of inflammatory cells (9, 10). HA is normally found within the joint cartilage and it is widely accepted that HA injection in the joint cavity, i.e. viscosupplementation, improves symptoms of OA (11-15). Balazs and Denlinger were the first to suggest high molecular weight HA intra-articular application in order to restore the viscoelastic properties of the autologous synovial fluid, providing pain relief and improving articular mobility (16). After this evidence, more than 20 compounds, which differ according to the molecular weight, HA concentration, chemical modification and volume of each unit, injection schedule and therapeutic claims have been introduced into the medical market (2). High molecular weight cross-linked HA has been widely used in clinical practice due to its high viscosity allowing a better lubrication and a stronger shock-absorbing affect. For instance, Hylan G-F 20 (Synvisc®) is a formaldehyde and divinyl sulfone cross-linked molecule composed of two hylan polymers within a buffered physiological NaCl solution with different rheological properties characterized by a viscosity and elasticity comparable with synovial fluid (16, 17).

In our aesthetic medicine clinic, we had a previous experience with Variofill®, a highly cross-linked HA formula characterized by a very high density. Variofill® is prepared from a 33 mg/ml non-cross-linked HA followed by an extended cross-linking process with divinyl sulfone and successive particularization of the strong cross-linked HA-gel mass and homogenization without dilution. The resulting compound is highly viscoelastic and easy to inject. In the field of aesthetic medicine, this compound guarantees long duration (up to 18 months) and restoration in the skin when used for body contouring as confirmed by previous histological studies conducted in in a rat model of subcutaneous biomaterial implantation in our laboratory (Fig. 1).

The aim of this pilot clinical study was to investigate the clinical effectiveness of viscosupplementation with the new highly cross-linked HA, Variofill®, in patients affected by bilateral knee OA, in comparison with the widely used Synvisc®.

MATERIALS AND METHODS

Synvisc®

Synvisc® (Hylan G-F 20) is a viscoelastic fluid containing hylans. Hylans are derivatives of HA sodium salt of avian origin. Synvisc® contains 80% Hylan A fluid and 20% Hylan B gel in buffered physiological sodium chloride solution (pH 7.2).

Variofill®

Variofill® is a gel of sodium hyaluronate purified from a streptococcus species of bacteria. It is chemically cross-linked and suspended in physiologic buffer at pH = 7 to a concentration of 33 mg/ml.

Patients

Forty-five patients (male = 26, female = 19) were screened to participate in this pilot randomized triple-blind clinical study. The inclusion criteria were bilateral knee OA (Kellgren-Lawrence grade II and III (18)), as diagnosed by Magnetic Resonance Imaging (MRI) and a minimum pain score $\geq$ 30 on both knees as assessed by Visual Analogue Scale (VAS; 0–100 mm, 0 = no pain, 100 = very severe pain). All patients signed the informed consent. The procedures were carried out at the Policlinico del Secondo Parere (Modena, Italy) according to the Helsinki declaration and local Internal Review Board (IRB) rules. Exclusion criteria were: patients with unilateral knee OA or unilateral/bilateral knee OA concerning predominantly the patellofemoral region; meniscal- or ligamentous-related instability, as assessed by physical examination; any prior viscosupplementation or intra-articular injection of corticosteroids or any other drug in the knee within 5 months prior to the beginning of the study; concomitance of other pathologies affecting the knee; anticoagulant therapy.

Methodology

VAS and Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) scores were used to evaluate the efficacy of HA injections before and 6 months after treatment. WOMAC is based on 5 items related to pain (subscore: 0-20; 0 = minimum pain subscore; 20 = maximum pain subscore), 2 to stiffness (subscore: 0-8; 0 = minimum stiffness subscore; 8 = maximum stiffness subscore) and 17 to physical activity (subscore: 0-68; 0 = minimum physical function subscore; 68 = maximum physical function subscore). Patients were advised not to use any analgesic drug 24 hours before baseline assessment.

With the patients comfortably lying in bed, 2 injections (2 ml each) were performed spaced 15 days apart. Variofill® injection was performed on their right knee while Synvisc® was injected into their left knee by the same surgeon who was blinded for the duration of the study and did not participate in the data evaluation. For ethical reasons, we decided to use Synvisc® as control, given that it is a widely used HA product for viscosupplementation for knee OA. Data were evaluated by a blinded allied health professional. The procedure is summarized as follows:

1) Epidermal anaesthesia with Emla cream (AstraZeneca, Milan, Italy) was performed 45 minutes before the procedure.

2) Disinfection with a 10% povidone-iodine (Meda Pharma Milan, Italy) was performed. The synovial cavity was approached from the lateral side with a 21 gauge syringe-recorded needle, in order to explore and drain a possible synovial fluid effusion or involuntary vascular lesion.

3) After arthrocentesis (performed if needed), the syringe with 2 ml of cross-linked HA (Synvisc® or Variofill®) was gently inserted into the cavity; the needle was quickly withdrawn at the end of the procedure; the patient was left in a supine position for 10 minutes whilst the joint was wrapped in an elastic bandage for 24 hours in order to help the distribution of an adequate amount of HA across the synovial surface exposed to major friction.

Following viscosupplementation, all patients were advised to avoid NSAIDs for 6 months, while paracetamol, at a maximum dose of 2000 mg/day, was allowed for pain management. All patients were advised to stop analgesics for 24 h before each assessment (3, 6 months).

Statistical analysis

Data were analyzed using GraphPad Prism 5.04. Change in VAS and WOMAC scores after Variofill® and Synvisc® injection were calculated as baseline – post-treatment at 3 and 6 months and compared using a 2 sample t-test. A one-way ANOVA was also used to compare the effect of Variofill® and Synvisc® treatment at 3 and 6 months *vs* baseline. A value of $p < 0.05$ was considered significant. All data are reported as mean ± Standard Error of the Mean (SEM).

RESULTS

Only 20 patients (males = 16; females = 4) aged between 24-74 (53.7±3.1; mean ± SEM) met the

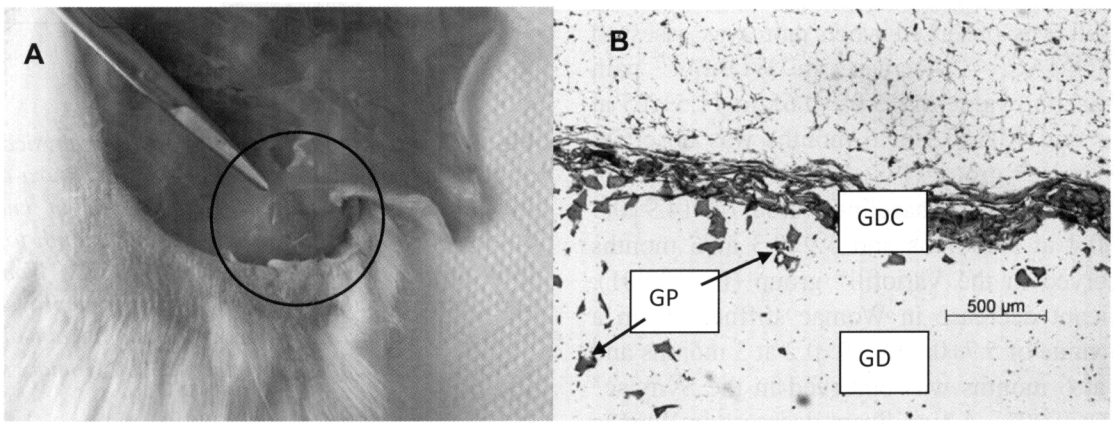

Fig. 1. *A) Variofill® gel 18 months after its subcutaneous implantation (0.25 ml) in rat hypodermis; **B**) Histological examination of thin gel depot capsula (GDC), gel depot (GD) and gel particle (GP) (outstanding long persistence of cross-linked HA in a rat model of biomaterial implantation).*

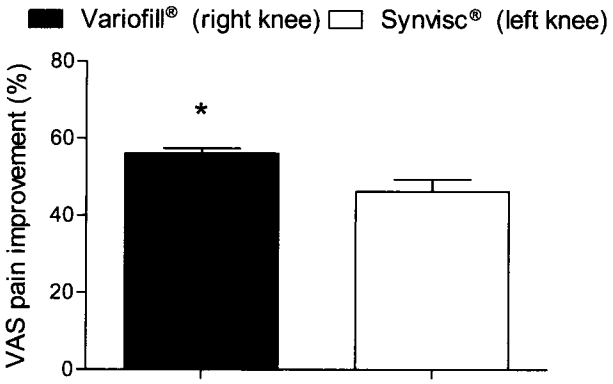

Fig. 2. *VAS pain improvement (%) following two intra-articular injections (2 ml each) of Variofill® (right knee) and Synvisc® (left knee) in 20 patients at 6-month follow-up. Data are presented as the group mean ± SEM. VAS pain was significantly improved in the right knee receiving Variofill®. *P<0.05 vs Synvisc®.*

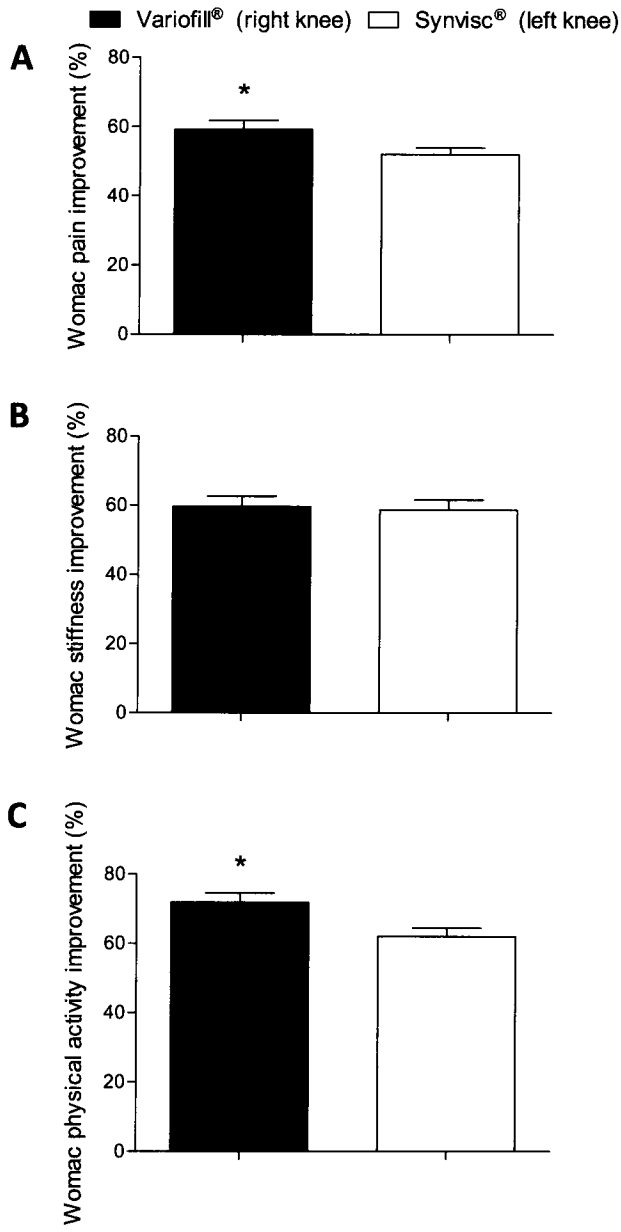

Fig. 3. *Womac pain (A), stiffness (B) and physical activity (C) improvement (%) following two intra-articular injections (2 ml each) of Variofill® (right knee) and Synvisc® (left knee) in 20 patients at 6-month follow-up. Data are presented as the group mean ± SEM. Womac pain and physical activity were significantly improved in the right knee receiving Variofill®. No significant difference between treatments was observed in Womac stiffness. *P<0.05 vs Synvisc®.*

inclusion criteria. They were randomized to receive Synvisc® on their left knee and Variofill® on their right knee. Variofill® and Synvisc® administration showed a significant reduction in VAS pain, WOMAC pain, physical activity and stiffness at 3 and 6 months *vs* baseline ($P < 0.001$) in knee OA patients. A decrease in VAS from a baseline value of 73.3±1.7 to 52.7±1.6 at 3 months and 39.3±2.2 at 6 months was observed in the Synvisc® group ($P < 0.001$ at all time points). A decrease in VAS from a baseline value of 74.7±1.5 to 53.4±1.4 at 3 months and 31.8±0.9 at 6 months was observed in the Variofill® group ($P < 0.001$). The same result was observed when pain was assessed using WOMAC. A decrease in WOMAC pain from a baseline value of 15.05±0.65 to 11.5±0.5 at 3 months and 7.05±0.3 at 6 months was observed in the Synvisc® group ($P < 0.001$). A decrease in Womac pain from a baseline value of 14.9±0.5 to 10.8±0.4 at 3 months and 5.9±0.3 at 6 months was observed in the Variofill® group ($P < 0.001$). A significant decrease in Womac stiffness from a baseline value of 5.7±0.2 to 3.9±0.2 at 3 months and 2.4±0.1 at 6 months was observed in the Synvisc® group ($P < 0.001$). A significant decrease in Womac stiffness from a baseline value of 6.2±0.2 to 4.1±0.2 at 3 months and 2.5±0.2 at 6 months was observed in the Variofill® group ($P < 0.001$). A decrease in WOMAC physical activity from a baseline value of 53.1±2.4 to 33.5±1.6 at 3 months and 19.6 ± 1.06 at 6 months was observed in the Synvisc® group ($P < 0.001$). A decrease in WOMAC physical activity

from a baseline value of 57.2±1.4 to 33.9±1.4 at 3 months and 15.8±1.05 at 6 months was observed in the Variofill® group ($P < 0.001$).

Inter-group analysis showed no significant difference between the two treatments at 3 months for VAS pain, WOMAC pain, stiffness and physical activity. At 6 months, Variofill® induced a significant percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity if compared to Synvisc® ($p < 0.05$ *vs* Synvisc® group; Figs. 2, 3A, 3C). No difference in percentage improvement in Womac stiffness between groups was observed (Fig. 3B). The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Variofill® group at 6 months was 56.94±1.18%, 59.54±2.55% and 72.84±3.32% respectively ($p < 0.05$ *vs* Synvisc® group). The percentage improvement in VAS pain, WOMAC pain and WOMAC physical activity in the Synvisc® group at 6 months was 46.2±3.1%, 52.02±1.9% and 62.003±2.4%, respectively. No serious adverse events were observed during treatment at all time points.

DISCUSSION

The present study is the first pilot trial designed to investigate the efficacy of Variofill® in patients affected by knee OA. Two injections of this compound, at a dose of 2 ml each and spaced 15 days apart, resulted in a high percentage improvement in VAS pain, WOMAC pain, and WOMAC physical activity when compared to Synvisc® viscosupplementation performed on the other knee. The second injection, performed 15 days after the first one, was supposed to add viscosupplementation bulk of HA, without filling the synovial cavity, which gives the patients unpleasant symptoms of fullness and motion limitation.

HA injections have been extensively used in patients affected by knee OA in order to achieve a significant improvement in OA-related symptoms including pain and ability to perform daily activities (3, 16).

This pilot randomized triple-blind clinical study, comparing two consecutive injections of Variofill® *vs* Synvisc®, has not shown any systemic side effects or local major untoward reactions. As a matter of fact, we knew in advance that Variofill® is a safe compound, as observed in our aesthetic medicine clinic. However, this is the first time that this compound is investigated in the orthopaedic field and the results are encouraging, despite the limited number of cases treated. The results of the present study can be explained by the greatest density of Variofill® due to its high cross-linking density and concentration, a more sustained coating and antifriction effect across the areas where the cartilage is fractured or damaged, achieving, during its degradation, a progressive lubricant and protecting effect on synoviocyte recovery. We suggest that the relented Variofill® turnover, especially in an acute or subacute inflammatory environment, accounts for a quicker functional knee reactivation with reduced pain. The results of our study can support Variofill® potential clinical use in patients affected not only by knee OA, but also in other different joints where the persistence of cross-linked HA is required notwithstanding the high pressure of the body weight over the cartilage, either at rest or while performing daily activities.

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