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ORIGINAL

Efficacy and safety of a single intra-articular injection of 2% hyaluronic acid and mannitol in knee osteoarthritis over a 6-month period

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Hyaluronic acid. Infiltration. Osteoarthritis. Chondroprotection

Efficacy and safety of a single intra-articular injection of 2% hyaluronic acid plus mannitol in knee osteoarthritis over a 6-month period

Abstract
Objective: To evaluate the safety and efficacy of a single intraarticular injection of 2% hyaluronic acid (HA) + mannitol in symptomatic knee osteoarthritis (KOA).
Material and methods: Pilot, multicentre, open, non-comparative study performed in eighty patients with painful KOA, of whom 79 completed the study. They received one 2 ml injection of 2% HA + 0.5% mannitol (Day 0) and were followed-up for 6 months. Pain and joint function were assessed on Days 0, 15, 30, 60, 90, 120, 150 and 180 using a visual analogue scale (VAS) and the WOMAC index. Efficacy and safety judgements by investigator and patients, and rescue medication intake, as an indirect measure of pain, were also recorded.
Results: A significant reduction in joint pain, stiffness and functional disability compared with baseline was observed at every follow-up visit ($p<0.001$). Joint function improved by 38.7% on Day 30, reaching 47.5% on Day 180. Rescue medication intake decreased from 58.2% at baseline to 2.5% on Day 90, increasing in the last visits. Efficacy and safety were positively evaluated by the investigators and patients. No serious adverse events were observed. Mild side effects were reported in 4 patients (local pain and swelling at the injection site).
Discussion: There is evidence that repeated intra-articular injections of HA improve symptoms in KOA. However, studies with a single injection of HA have shown mixed results. This study demonstrates that a single intra-articular injection of non-crosslinked HA reduces joint pain and increases function in patients with KOA over a period of at least 6 months.

INTRODUCTION

Osteoarthritis is a disease that affects the synovial joints and is characterized by the degradation and loss of articular cartilage with subchondral bone remodelling, osteophyte formation and inflammation of the synovial membrane. Clinical signs include fluctuating joint pain, swelling, stiffness and loss of mobility, which increases in severity as the disease progresses[1-3]. It is one of the most common causes of long-term disability among adults[4-7].

Given the lack of a curative agent, the main treatment goals in osteoarthritis are currently to reduce symptoms, minimize functional impairment and limit the progression of structural changes[1-4,8]. Current treatment options include non-pharmacological measures such as weight loss, the use of assistive devices, exercise and physiotherapy; pharmacological measures include the use of analgesics or non-steroidal anti-inflammatory drugs, SYSADOAs (slow-acting drugs for the symptomatic treatment of osteoarthritis which include glucosamine, chondroitin sulphate and diacerein), opioids, intra-articular (i.a.) injections of corticosteroids and of hyaluronic acid (HA) and, in more advanced stages, surgical treatment[3-5,8-10].

Intra-articular HA is a widely used treatment to improve pain and joint function[4,9,11]. It is an endogenous, high molecular weight glycosaminoglycan and is distributed throughout the body, mainly in the hyaline cartilage, the synovial fluid of joints, the skin, vitreous humor and the connective tissue of soft tissue[8,9]. HA lubricates synovial joints, provides shock absorption, stabilizes the structure and has direct effects on the function of the synovial cells[8,9].

In arthritic joints the synovial fluid contains a lower concentration of HA than in healthy joints[3,8-10], which causes a substantial reduction in its viscoelasticity thus decreasing its lubricating and shock-absorbing functions[7,9]; this increases the mechanical load on the joint and causes changes in the cartilage[7], subchondral bone and the synovial membrane. These changes ultimately produce pain and functional impairment of the affected joint. As the elasticity and viscosity of the synovial fluid are directly proportional to the content and integrity of HA therein, an i.a. injection of HA is a rational approach to the treatment of osteoarthritis[8,9,11]. It has been used successfully in degenerative processes of articular cartilage, through direct i.a. injection in order to enhance the activity of synovial fluid and, as a result, joint function[12-14]. In addition, several clinical trials have shown that repeated i.a. injections of HA at different doses improves symptoms, especially pain, in osteoarthritis[7,11,14-18].

Nevertheless, i.a. injections of HA can cause adverse effects, some of which are related to the origin of the product (obtained from animal protein such as rooster combs) and can be attributed to biological impurities[8]. Other adverse effects associated with the infiltration of HA, such as pain and swelling, are related to the high molecular weight and high concentration of some available pharmaceutical specialties of semi-synthesised, cross-linked HA[11] (HA chains synthetically stabilized by cross-linking). Given that multiple injections (3-5) are required to achieve the desired efficacy with most of the HA products on the market (due to their rapid degradation in the joint[8,16]), the stabilization of the HA and the resultant increase in residence time in the joint, enables the number of injections required to achieve long-term efficacy in the treatment of osteoarthritis to be reduced[6-8]. A single i.a. injection of HA may represent an alternative to current treatment

regimens in terms of tolerability, logistics and costs, due to a fewer number of injections and fewer visits to the physician, thus offering greater well-being and safety to patients by reducing the risks associated with repeated infiltrations, as well as economic and logistic benefits for the hospital or medical centre.

The main objective of this study was to assess the long-term efficacy of Ostenil plus® (Laboratorios Masterfarm S.L., Barcelona, Spain), in relieving pain and improving joint function. Ostenil plus®, a transparent 2ml solution of natural and highly purified 2% sodium hyaluronate obtained by fermentation and devoid of animal proteins, also contains 0.5% mannitol, a free radical scavenger which helps to stabilize the sodium hyaluronate chains thus increasing their residence time in the joint without increasing its molecular weight. The primary objective was to assess the effects of a single i.a. injection of HA on the symptoms of knee osteoarthritis. The secondary objective was to assess and define the safety of the product by evaluating its tolerability and monitoring adverse effects.

MATERIALS AND METHODS

We carried out a pilot, exploratory, prospective, open, non-comparative, multicentre, phase IV study. It was conducted at the Departments of Orthopaedic Surgery and Traumatology (COT) of the following centres: the Virgen Macarena University Hospital, Seville; the Prince of Asturias University Hospital, Madrid; and the Virgen de la Arrixaca University Hospital, Murcia.

Eighty patients (aged 40 years and over) diagnosed with grade III knee osteoarthritis according to the *American College of Rheumatology (ACR)* criteria were included in the study. The inclusion criteria were: patients with at least grade III joint function in the knee to be treated, diagnosed according to the ACR criteria (radiographs, symptoms and signs), and who had suffered pain and discomfort in the affected knee for most days in the last 3 months. Excluded from the study were patients suffering from other diseases that could confound or interfere with the efficacy assessments, those who had received i.a. injections of steroids and/or HA in the last 180 days, patients who had undergone an arthroscopic joint lavage in the last year or who were taking oral chondroprotective agents such as glucosamine or chondroitin sulfate, or supplements of enzymatically-hydrolysed collagen in the 2 months prior to study start, patients who had participated in another clinical trial in the last 30 days and pregnant women. Prior to participation in the study, all subjects signed an informed consent form which was approved by the Clinical Research Ethics Committees of each of the previously mentioned study centres.

A control group was not included in the study design as the aim of this study was to evaluate the long-term efficacy of a single infiltration of non-cross-linked HA plus mannitol, and because the efficacy of HA infiltrations has been previously demonstrated.

The 80 patients received one intraarticular injection of 2% sodium hyaluronate + 0.5% mannitol (Ostenil Plus®) at the first visit and were monitored for 6 months, with eight assessments visits on days 0, 15, 30, 60, 90, 120, 150 and 180. The primary efficacy parameters evaluated were the clinical evolution of pain and joint function, measured using a 10 cm visual analogue scale (VAS) for pain and the *Western Ontario and McMaster Universities Osteoarthritis* (WOMAC) scale to measure pain and joint function (stiffness and physical function), parameters that have an effect on the physical function and quality of life of the affected patients. Also assessed were the judgements by the physician and the patients regarding the efficacy and tolerability of the treatment and the possible occurrence of both local and systemic undesirable effects was monitored. Moreover, during the study patients were allowed to take 1 g paracetamol and/or 400 mg ibuprofen up to a maximum of 3 g paracetamol or 1200 mg ibuprofen, provided that knee pain was greater than, or equal to, 7 cm on the VAS, according to the subjective assessment by the patient. The intake of rescue medication was also recorded and analyzed as an indirect measurement of pain, considering whether the intake was regular, sporadic or did not occur.

The statistical analysis was based on an intention-to-treat (ITT) analysis. The ITT analysis was performed on the final data recorded for each patient.

The data were analysed using IBM SPSS 19.0 for Windows. A descriptive statistical analysis of all the variables analysed in the study (n, mean, standard deviation and graphs with mean and 95% confidence intervals of the mean for each of the variables) was carried out. Concerning inferential statistics, an analysis of variance for repeated measures (linear mixed model) was carried out to analyse the evolution of different variables across visits. 2 x 2 comparisons were carried out versus control with a Bonferroni correction. The first visit was considered as the control visit for the variables VAS and WOMAC, while the second visit was considered as the control visit for the global efficacy and tolerability judgements of the treatment expressed by the physician and the patients. The confidence level ($1-\alpha$) was set at 95%, with a significance level of 0.05 and a statistical power of 90%.

RESULTS

A total of 80 patients were included into the study. One patient was excluded from the study for not presenting at the follow-up visits after the treatment was administered. Thus the analysis included 79 evaluable patients who continued the follow-up period. Of the 79 patients, 6 dropped out during the follow-up period for reasons not related to the study: 2 patients discontinued at the third and sixth visit, respectively, due to traumatic accidents, while 4 patients dropped out of the study as they did not return for the final assessment visits. For statistical analysis, the final data obtained for these patients were carried forward to the end of the study, as stipulated in the protocol.

Concerning the primary efficacy parameters assessed, the mean joint pain, measured using the VAS, showed a statistically significant decrease ($p<0.001$) from the first follow-up visit (day 15)

Table 1

Visit	Total WOMAC	WOMAC Pain	WOMAC Stiffness	WOMAC Physical function
1	2.302 ± 0.597	2.308 ± 0.660	2.227 ± 1.027	2.309 ± 0.599
2	1.664 ± 0.773*	1.625 ± 0.758*	1.487 ± 1.043*	1.696 ± 0.781*
3	1.411 ± 0.788*	1.357± 0.756*	1.196 ± 0.871*	1.453 ± 0.813*
4	1.308 ± 0.817*	1.253 ± 0.784*	1.113 ± 0.891*	1.347 ± 0.847*
5	1.276 ± 0.826*	1.227 ± 0.776*	1.107 ± 0.911*	1.310 ± 0.861*
6	1.240 ± 0.807*	1.212 ± 0.763*	1.044 ± 0.855*	1.271 ± 0.840*
7	1.233 ± 0.781*	1.192 ± 0.733*	1.075 ± 0.877*	1.264 ± 0.813*
8	1.209 ± 0.703*	1.146 ± 0.659*	1.031 ± 0.805*	1.248 ± 0.739*

WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.
Mean ± standard deviation for the WOMAC scale (total, pain, stiffness and physical function).
Statistically significant differences (*p <0.001) for 2 x 2 comparisons for the first visit (Bonferroni method).

compared to baseline (before i.a. infiltration of HA), with the decrease being maintained up to the final visit (6 months). Figure 1 shows the differences in pain in the knee joint from the start of the study, where the mean value was 7.41 (out of 10), until the end, when it reached a mean value of 3.97.

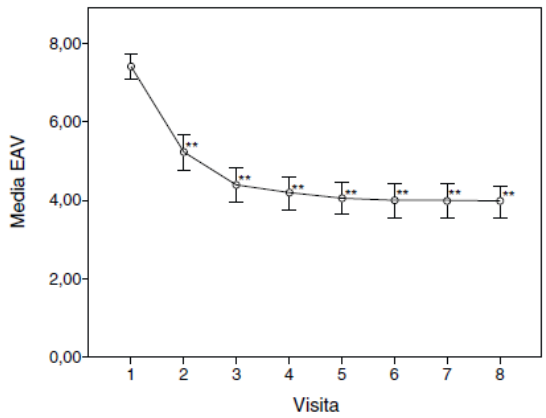

Figure 1: Evolution of mean joint pain (VAS). 95% confidence intervals. Statistically significant differences (**p<0.001) for 2 x 2 comparisons compared to the baseline visit (Bonferroni method).
(Translation: Media EAV = mean VAS; Visita = Visit)

In addition, the quality of life assessment, namely pain and joint function, measured using the WOMAC index (where 0 = none; 4 = extreme), showed a statistically significant decrease (p<0.001) from the second visit onwards, compared to the baseline visit, whether the total value or the individual values for the components pain, stiffness and functional impairment were considered. This decrease was maintained at the subsequent visits up to 6 months after treatment. Table 1 summarizes the changes in the WOMAC scale at the different visits. The percentage of patients that presented improved joint function was also calculated from the total WOMAC index values (Figure 2). It was observed that at 30 days after treatment, joint function had improved by 38.7% compared with its baseline value, reaching an improvement of 47.5% at 180 days.

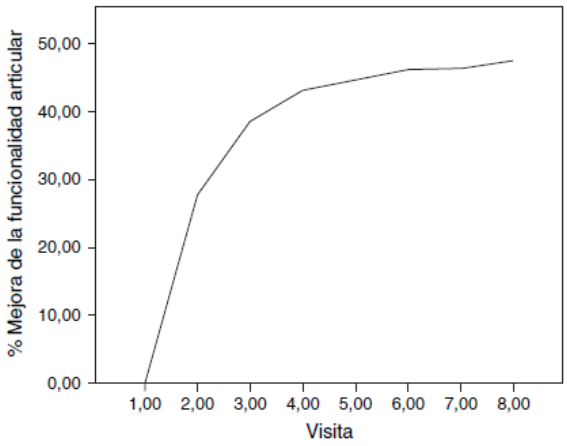

Figure 2: Percentage improvement in joint function during the visits (using the Total WOMAC scale)
(Translation: % Mejora de la funcionalidad articular = % improvement in joint function; Visita = Visit)

Moreover, the mean efficacy judgement both by the investigator and the patients, and scored from 0 (worst) to 4 (ideal), was good or very good throughout the study. There were statistically significant differences (p<0.05) between the first efficacy assessment (second visit), and subsequent assessments in all cases except for the patient judgement at the final visit, which showed no significant difference compared to the baseline value. This final assessment by the patients suggests that some of the initial symptoms begin to re-appear at 6 months after the start of treatment. These results are presented graphically in Figure 3.

Figure 4 summarizes the data on the intake of rescue medication throughout the study. At the initial study visit, most patients (58.2%) regularly took analgesics and anti-inflammatory drugs and this intake decreased considerably as the study progressed such that at visit 5 (day 90) regular intake was lower (2.5%). Nevertheless, both regular and sporadic intake tended to increase from visits 6 and 7 reaching 17.7% of regular intake of paracetamol and/or ibuprofen at the final visit.

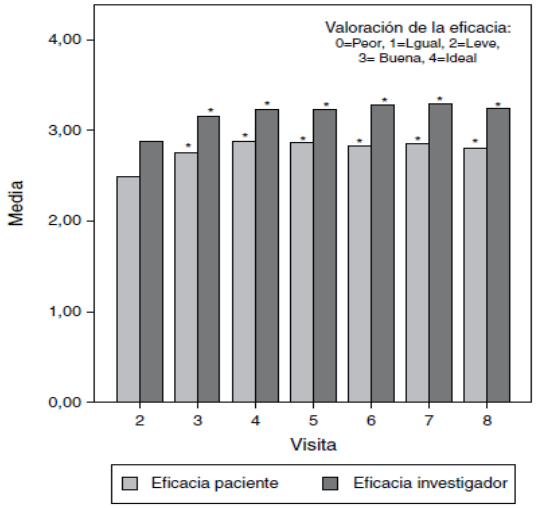

Figure 3: Evolution of mean values for treatment efficacy, evaluated by the investigator and the patient. Statistically significant differences (*p<0.05) for 2 x 2 comparisons compared to the second visit (Bonferroni method). (Translation: Media = Mean; Visita = Visit; Valoración de la eficacia: 0 = Peor, 1 = Lgual, 2 = Leve, 3 = Buena, 4 = Ideal; Evaluation of the efficacy : 0 = Worse, 1 = unchanged, 2 = slight, 3 = Good, 4 = Ideal; Eficacia paciente = Efficacy patient; Eficacia investigador = Efficacy investigator).

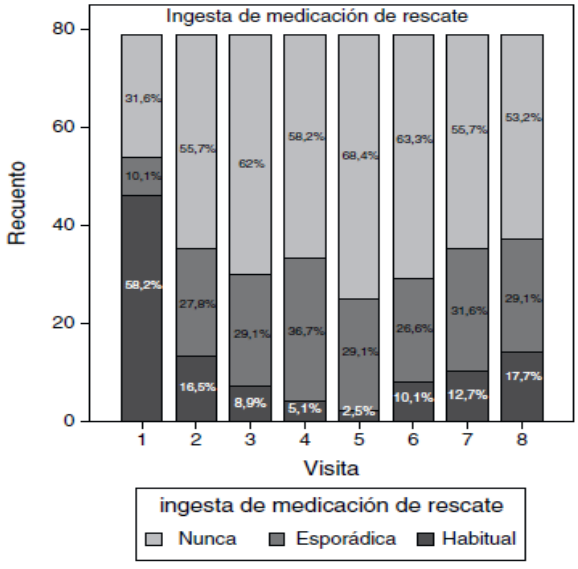

Figure 4: Evolution of paracetamol and/or ibuprofen intake during the visits. (Translation: Ingesta de medicación de rescate = intake of rescue medication; Nunca = None; Esporádica = Sporadic; Habitual = Normal; Recuento = Count; Visita = Visit).

For the evaluation of tolerability, both the investigator and the patients scored their tolerability assessment at each visit. No statistically significant differences were observed throughout the study which indicates that tolerability was excellent from the start of the study (infiltration visit) up to the end.

Concerning safety, no serious adverse effects were observed during the study. Mild adverse effects were reported in 5.06% of patients (n = 4) at the second follow-up visit. These were mild adverse events in all cases and consisted of mild pain and inflammation at the injection site. These effects disappeared at subsequent visits.

DISCUSSION

At present, many of the HA pharmaceutical specialities available on the market are administered in repeated doses (3 to 5 injections) and several clinical studies have demonstrated that this improves symptoms, especially pain, in osteoarthritis[4,10,11,14]. More recently, clinical studies carried out in order to demonstrate the efficacy and safety of a single injection of HA in the treatment of osteoarthritis of the knee and hip gave mixed results[7,8,19,20]. In most cases, these were studies with high molecular weight HA formulations (cross-linked or semi-synthetized in the laboratory), where the results obtained demonstrate efficacy, with mixed results for the duration of response and safety, and with more mild local adverse effects compared to chemically unmodified HA (not cross-linked)[7,20]. Only in one study, conducted by Richette et al.[6], was a single injection of chemically unmodified, medium molecular weight HA, obtained by fermentation, infiltrated in patients with osteoarthritis of the hip and the results obtained after 3 months follow-up were not satisfactory as there was no difference in pain reduction between the placebo and treated groups. Different hypotheses, such as the high placebo effect, the study design or the lack of efficacy of the treatment itself due to the concentration and/or dose administered, could explain the lack of efficacy of a potentially active treatment in a clinical trial.

Considering the above, this study was designed with the aim of administering a single i.a. injection of HA for specific reasons. Firstly, performing repeated injections could lead to an increased risk of local adverse effects. Secondly, reducing the number of injections and visits to the physician is a major convenience for the patient and an economic and logistic advantage for the hospital or medical centre. And thirdly, there are no recent studies in knee osteoarthritis where a single injection of non-crosslinked, medium molecular weight HA is performed and where safety and efficacy results are assessed in the long term. Therefore, a single i.a. injection of HA may represent a therapeutic alternative to current treatment regime in terms of efficacy, safety and comfort for the patient and logistics for the medical centre.

The results obtained in this study demonstrate that a single i.a. injection of 2% HA + 0.5% mannitol (Ostenil plus®) is effective in reducing pain in the long-term in patients with knee osteoarthritis. Its specific composition, which contains mannitol, increases the stability of the HA and its salts when injected intra-articularly, thus prolonging the mean residence time of HA in the joint cavity by protecting it from degradation[21,22]. The mean values of the efficacy assessments (VAS and WOMAC Index) show a clear and statistically significant improvement after treatment for all parameters evaluated, including those that directly refer to pain and those related to joint function and the quality of life. Moreover, this statistically significant (p<0.001) improvement was maintained throughout the study (6 months).

The same parameters were assessed in similar studies using questionnaires, such as the Lequesne index[10], which is different from the WOMAC index. In this study we decided to use the WOMAC index as it is a specific and validated instrument for osteoarthritis, and is useful to clinically assess pain, joint stiffness and functional capacity of the affected patients. The Lequesne index was developed to evaluate the severity of hip osteoarthritis, but there is a specific version for the knee, and its assessments include pain, maximum walking distance and daily activities.

Despite the limitations of the study (open, non-comparative study design), these efficacy results establish that the therapeutic effect of the treatment persists during the 6 months follow-up period. This finding is reinforced by data collected from the records of rescue medication intake by the patients, which show that after 6 months some of the treated patients started sporadic use of analgesics and anti-inflammatory drugs without, however, reaching their intake level at baseline. At this point, a clinical follow-up of the patients is required to decide when the treatment should be repeated.

Another objective of the study was to evaluate the safety profile of the treatment. The treatment was very well tolerated as underlined by the tolerability judgements expressed by the investigators and the patients and by the low incidence (5.06%) of adverse events during the study. These results contrast with those obtained in earlier studies with high molecular weight HA formulations (cross-linked or obtained by semi-synthesis), which showed a high incidence of pain and swelling at the injection site in the days after injection[7,20]. The excellent safety profile of treatment translates into a good benefit/risk ratio for the patient.

In conclusion, this is the first study to have demonstrated that a single i.a. injection of non-cross-linked 2% HA + 0.5% mannitol is an effective treatment for osteoarthritis of the knee as it decreased pain and improved joint function for a minimum period of 6 months and also presented a low incidence of associated mild adverse effects.

In daily practice, the favourable benefit/risk ratio of a single i.a. injection of 2 ml HA 2% + mannitol is a good therapeutic option to reduce the number of HA injections from between three and five injections per treatment cycle to only one injection per treatment cycle. Larger studies are needed to determine the duration of a treatment cycle greater than 6 months follow-up.

Level of Evidence

Level of Evidence III.

Ethical responsibilities

Protection of people and animals. The authors declare that for this investigation no experiments were performed on humans or animals.

Data confidentiality. The authors declare that they have followed the protocols in force at their study centres on the publication of patient data, and that all the patients included in the study had received sufficient information and gave their written informed consent to participate in the study.

Right to privacy and informed consent. The authors have obtained informed consent from the patients and/or subjects referred to in the article. This document is held by the corresponding author.

Conflict of interest

The authors declare that they have no conflict of interest.

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BIBLIOGRAPHY

1. Pelletier JP, Martel-Pelletier J. Therapeutic targets in osteoarthritis: from today to tomorrow with new imaging technology. Ann Rheum Dis. 2003;62 Suppl 2:ii79-82.

2. Pavelka K, Trc T, Karpas K, Vitek P, Sedlácková M, Vlasáková V *et al.* The efficacy and safety of diacerein in the treatment of painful osteoarthritis of the knee: a randomized, multicenter, double-blind, placebo-controlled study with primary end points at two months after the end of a three-month treatment period. Arthritis Rheum. 2007;56:4055-64.

3. Frampton JE. Hylan G-F 20 single-injection formulation. Drugs Aging. 2010;27:77-85.

4. Sun SF, Hsu CW, Hwang CW, Hsu PT, Wang JL, Tsai SL *et al.* Hyaluronate improves pain, physical function and balance in the geriatric osteoarthritic knee: a 6-month follow-up study using clinical tests. Osteoarthritis Cartilage. 2006;14:696-701.

5. Lundsgaard C, Dufour N, Fallentin E, Winkel P, Gluud C. Intra-articular sodium hyaluronate 2 mL versus physiological saline 20 mL versus physiological saline 2 mL for painful knee osteoarthritis: a randomized clinical trial. Scand J Rheumatol. 2008;37:142-150.

6. Richette P, Ravaud P, Conrozier T, Euller-Ziegler L, Mazières B, Maugars Y *et al.* Effect of hyaluronic acid in symptomatic hip osteoarthritis: a multicenter, randomized, placebo-controlled trial. Arthritis Rheum. 2009;60:824-30.

7. Chevalier X, Jerosch J, Goupille P, van Dijk N, Luyten FP, Scott DL *et al.* Single, intra-articular treatment with 6 ml hylan G-F 20 in patients with symptomatic primary osteoarthritis of the knee: a randomised, multicentre, double-blind, placebo controlled trial. Ann Rheum Dis. 2010;69:113-9.

8. Altman RD, Akermark C, Beaulieu AD, Schnitzer T, Durolane International Study Group. Efficacy and safety of a single intra-articular injection of non-animal stabilized hyaluronic acid (NASHA) in patients with osteoarthritis of the knee. Osteoarthritis Cartilage. 2004;12:642-9.

9. Strauss EJ, Hart JA, Miller MD, Altman RD, Rosen JE. Hyaluronic acid viscosupplementation and osteoarthritis: current uses and future directions. Am J Sports Med 2009; 37: 1636-44.

10. McDonald C, Hantel S, Strohmeier M. A randomised, controlled study to compare the performance and safety of two sources of sodium hyaluronate given as a viscosupplement by intra-articular injection to patients with osteoarthritis of the knee. J Clin Res. 2000;3:41-50.

11. Tikiz C, Unlü Z, Sener A, Efe M, Tüzün C. Comparison of the efficacy of lower and higher molecular weight viscosupplementation in the treatment of hip osteoarthritis. Clin Rheumatol. 2005; 24:244-50.

12. Van Den Bekerom MP, Mylle G, Rys B, Mulier M. Viscosupplementation in symptomatic severe hip osteoarthritis: a review of the literature and report on 60 patients. Acta Orthop Belg. 2006;72:560-8.

13. Fernández López JC, Ruano-Ravina A. Efficacy and safety of intraarticular hyaluronic acid in the treatment of hip osteoarthritis: a systematic review. Osteoarthritis Cartilage. 2006;14:1306-11.

14. Altman RD, Rosen JE, Bloch DA, Hatoum HT, Korner P. A double-blind, randomized, saline-controlled study of the efficacy and safety of EUFLEXXA for treatment of painful osteoarthritis of the knee, with an open-label safety extension (the FLEXX trial). Semin Arthritis Rheum. 2009;39:1-9.

15. Balazs EA, Denlinger JL. Viscosupplementation: a new concept in the treatment of osteoarthritis. J Rheumatol Suppl. 1993;39:3-9.

16. Peyron JG. Intraarticular hyaluronan injections in the treatment of osteoarthritis: state-of-the-art review. J Rheumatol Suppl. 1993;39:10-5.

17. Wollheim FA. Current pharmacological treatment of osteoarthritis. Drugs. 1996;52 Suppl 3:27-38.

18. Altman RD, Moskowitz R. Intraarticular sodium hyaluronate (Hyalgan) in the treatment of patients with osteoarthritis of the knee: a randomized clinical trial. Hyalgan Study Group. J Rheumatol. 1998; 25:2203-2212.

19. Krocker D, Matziolis G, Tuischer J, Funk J, Tohtz S, Buttgereit F *et al.* Reduction of arthrosis associated knee pain through a single intra-articular injection of synthetic hyaluronic acid. Z Rheumatol. 2006;65:327-31.

20. Conrozier T, Couris CM, Mathieu P, Merle-Vincent F, Piperno M, Coury F *et al.* Safety, efficacy and predictive factors of efficacy of a single intra-articular injection of non-animal-stabilized-hyaluronic-acid in the hip joint: results of a standardized follow-up of patients treated for hip osteoarthritis in daily practice. Arch Orthop Trauma Surg. 2009;129:843-8.

21. Mendoza G, Alvarez AI, Pulido MM, Molina AJ, Merino G, Real R *et al.* Inhibitory effects of different antioxidants on hyaluronan depolymerization. Carbohydr Res. 2007;342:96-102.

22. Di Napoli G. Composition and method for intradermal soft tissue augmentation. Aventis Pharmaceuticals Holding September 2004. WO 2004/073759.