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ORIGINAL ARTICLE

The effects of an oral preparation containing hyaluronic acid (Oralvisc®) on obese knee osteoarthritis patients determined by pain, function, bradykinin, leptin, inflammatory cytokines, and heavy water analyses

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Abstract The purpose of this study was to determine the effects of an oral preparation containing hyaluronic acid on osteoarthritic knee joint pain and function as well as changes in inflammatory cytokines, bradykinin, and leptin. We also used heavy water to determine the turnover rates of glycosaminoglycans in synovial fluid. This was a double-blind, randomized, placebo-controlled study of 40 subjects over a period of 3 months. Visual analog scale, Western Ontario McMaster pain, and WOMAC function scores were recorded. Serum and synovial fluid were measured by enzyme-linked immunosorbent assays for inflammatory cytokines, bradykinin, and leptin. In 20 subjects, terminal heavy water ingestion was used for spectral analyses of serum and joint fluid samples. There were statistically significant improvements in pain and function. Both serum and synovial fluid samples showed significant decreases for a majority of inflammatory cytokines, leptin, and bradykinin in the oral hyaluronic acid preparation group. Heavy water analyses revealed a significant decrease in hyaluronic acid turnover in the synovial fluid of the treatment group. A preparation containing hyaluronic acid and other glycosaminoglycans holds promise for a safe and effective agent for the treatment for patients with knee osteoarthritis and who are overweight. Further studies will be required to see whether this is a disease-modifying agent.

Keywords Osteoarthritis · Clinical Outcomes Research · Knee · Cytokines · Pain · Function

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Introduction

Osteoarthritis (OA) is a multifaceted disease characterized by the loss of homeostasis in articular cartilage due to mechanical and other biologic factors that lead to a wide variety of metabolic and physical changes throughout the joint [1]. It is a major source of disability, loss of income, and independence [2, 3]. On an annual basis during the years 1996 to 2005, the medical cost for OA was $185.5 billion. Of that amount, out-of-pocket expenditures were $36.1 billion [4]. The work loss costs are staggering [5]. Knee OA predominates in producing disability and need for surgery [6].

Among the known risk factors for OA are age, significant trauma, obesity, altered gait, altered biomechanics (e.g., varus or valgus deformity), and excessive loading [7]. The connection between obesity and increased loading is mechanical given the relative reduced activity level among obese individuals. However, obesity is associated with metabolic syndrome (MS). MS is a combination of hypertension, diabetes, dyslipidemia, and obesity [8]. MS is prevalent in OA patients and related to the effect of diabetes in this population [9, 10].

The predominant cause of physical dysfunction is pain. There are no pain receptors in articular cartilage, but there are pain receptors in bone, synovium, and menisci [11]. There are multiple neurotransmitters for pain receptors in these tissues including prostaglandins, acetylcholine [12], serotonin [12], gamma-aminobutyric acid [13], substance P [14], adenosine triphosphate [12], excitatory amino acids [15], and bradykinin [12]. Bradykinin is thought to be involved in initial injury pain transmission [16]. However, chronic elevations may be associated with increased pain [17].

The ability of cartilage to withstand joint loading is determined by the composition and structure of its dense extracellular matrix [18]. In the course of joint degeneration, there is a net loss of matrix molecules, such as the chondroitin sulfate (CS) chains that are critical to the ability of cartilage to withstand the load. Previous studies have attempted to compare the ability of "normal" and "osteoarthritic" cartilage to maintain or regenerate extracellular matrix by assessing donor tissue in various in vitro culture configurations [19]. However, it is difficult to extrapolate in vitro results to the in vivo scenario [20]. Evidence of articular cartilage degeneration is found in the synovial fluid (SF) where matrix molecules can accumulate in OA [1]. However, it has not been possible to know whether molecules removed from articular cartilage are more or less recently synthesized. In the normal and degenerate joint, articular cartilage interacts with multiple surrounding tissues including the joint capsule, bone, and SF, whose influence on the disease phenotype cannot be neglected. Hyaluronic acid (HA) has a defined and essential role in the biomechanics of normal SF, where it is partially responsible for the lubricating and viscosity properties of the SF [21]. In end-stage knee OA, HA concentration is significantly decreased [22]. The metabolic processes underlying these degenerative changes are poorly understood in vivo, but they could represent attractive targets for disease-modifying therapies that address degeneration rather than pain.

Stable isotopes such as heavy water (^2H_2O) provide a safe, effective option for measuring metabolic pathways in humans [23]. Using ^2H_2O for metabolic labeling, investigators have measured mammary epithelial cell proliferation [24], triglyceride synthesis [25], and protein synthesis [26, 27] in humans by analyzing the incorporation of deuterium into newly synthesized molecules. The approach has been recently extended to quantify extracellular matrix metabolism in the knee joints of rats [28].

Our purpose was to evaluate the effects of an oral preparation containing HA and other glycosaminoglycans (GAGs) patented for its use in treating OA. The trial's primary objective was to determine the effects of oral GAGs on both components of the WOMAC osteoarthritis index, the WOMAC pain score, and the WOMAC function score.

Parallel to this, we also wished to determine whether there were specific cytokine changes affected by treatment. This is particularly important given the relationship to MS and changes in inflammatory cytokines and chemokines. It is known that one of the adipokines, leptin, is associated with obesity and knee OA [29]. Our purpose was to evaluate the changes in leptin relative to the oral preparation. Additionally, we used ^2H_2O to determine the effect of the oral preparation on the kinetics of HA and CS in SF of OA patients to determine whether there was a biological component to the treatment response.

Our hypotheses were as follows:

1. Oral supplementation with oral GAGs during a period of 3 months improves pain and function in OA patients.
2. MS is related to pain and biochemical response to oral GAGs.
3. Twelve-week changes in serum and SF levels of inflammatory cytokines, chemokines, leptin, and bradykinin would vary from oral preparation to placebo.
4. The use of ^2H_2O will detect metabolic changes in serum and SF that vary from oral preparation to placebo.

Methods

With institutional review board (IRB) approval, 576 patient charts were reviewed for study eligibility at the time of a routine outpatient visit for knee OA. Knee OA was documented by any articular cartilage degradation or response to degradation, such as osteophytes, seen on imaging methods including magnetic resonance imaging (MRI) and radiographs. All patients had symptoms for at least 10 months. The subject population included adult male and female subjects between the ages of 50–75 years regardless of ethnicity, social, and economic background. All subjects had to have knee pain (>50 mm of visual analog scale (VAS)) and an effusion where a joint aspiration or intra-articular injection would be clinically indicated. All were intellectually competent to follow specific daily routines. The goal was to recruit 40 total patients with blind randomization to 20 patients receiving daily 80 mg of Oralvisc®, (Bioiberica, Barcelona, Spain) and 20 patients receiving same size and shape placebo. Oralvisc contains HA (70 %) and GAGs. The 80-mg dose was based on preclinical trials. None of the patients or investigators were aware of which group the patient was in until the study was completed. Ten patients in each group were given ^2H_2O two days prior to completion.

Clinical data

On the day of the visit, the exclusion of patients was determined by a senior investigator. Exclusion included recent trauma, any inflammatory joint disorder, such as crystalline disease and autoimmune disorders, pregnancy, intra-articular corticosteroid injections or oral treatment with corticosteroids within 4 weeks, intra-articular radioisotope injections within 3 months, intra-articular HA injections within 6 months, and topical treatment with corticosteroids within 1 week. Additional exclusions were trauma within 6 months, osteotomy on the study knee, arthroscopy within 3 months, any clinically significant medical or surgical condition (other than knee OA) that in the investigator's or sponsor's opinion would compromise the outcome of the study, and allergy to chickens, although no chicken products were used in the extraction process.

For blinding, unmarked boxes containing either oral preparation or placebo were randomized by a computer-generated randomization number. The randomization code was maintained by the sponsor and concealed from the study site. Treatment allocation depended only on the time sequence in which patients entered the study, thus minimizing selection bias. All study-related case report forms recorded only the randomization number with unblinding only after the completion of the study or analyses.

After meeting eligibility, 51 subjects were recruited between April and December 2011. Consent included feedback to make certain they were aware of the components and the 12-week length of this study. Two drug patients did not return; one had an adverse event (rash) and one had surgery. Three placebo patients did not return, and three had enrollment errors. All patients took their preparation daily for 3 months. This study was conducted for only 3 months since our interest was in the initial effect of the preparation and not long-term response or washout times. Twenty-one of the completing patients had been randomized to oral preparation and 19 to placebo. Two of the placebo patients could not have fluid withdrawn at the end of the study but all other data including serum samples were available (Fig. 1).

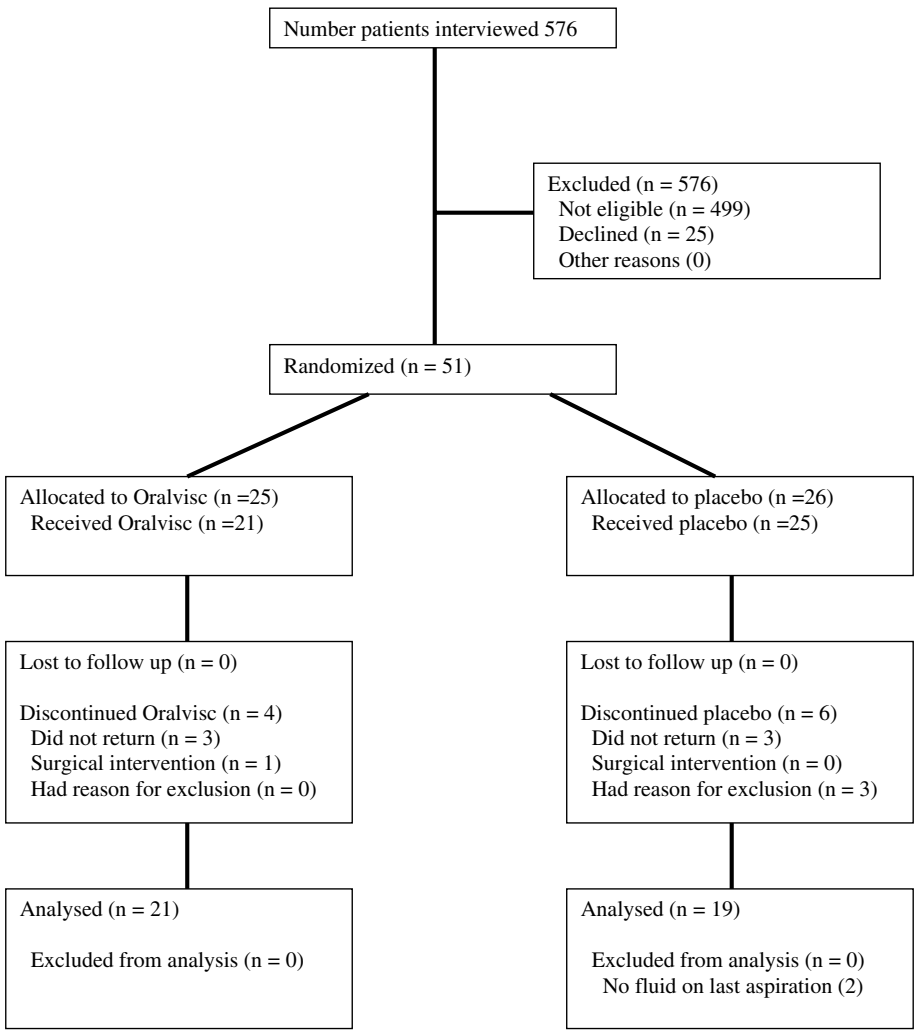

Fig. 1 Disposition of subjects

Patients were assessed each month for any unused capsules and received capsules for the next 4 weeks. Initial body mass index (BMI) was based on height and weight. The metabolic score components (hypertension, dyslipidemia, diabetes, and BMI) were scored 0–4, MRI changes, Kellgren–Lawrence (KL) scores, age, race, and sex were recorded. There were no restrictions on other pain medications or therapies. The 10 cm measured VAS score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score (0–20), and WOMAC function (0–80) were obtained during the initial and 3 monthly visits.

Initially, at least 5 mL of SF was withdrawn, spun, and saved. Additionally, a 5 mL sample of blood was obtained and processed to serum. SF and serum were frozen at −70 °C for later aliquot assortment.

The kit used for initial and 12-week serum and SF leptin and bradykinin was an immunoassay (Alpco, Salem, NH). Two patients could not have SF obtained leaving 21 oral GAGs and 17 placebo patients. The kit used for chemokines and cytokines was from a human inflammatory cytokines multi-analyte ELISArray Kit: MEH-004A (Qiagen, Valencia, CA).

At 12 weeks, 10 patients in each group received written and oral instructions for six vials of ^2H_2O (each containing 50 mL 70 % ^2H_2O) and two specialized saliva sampling kits (salivettes), consisting of a nylon-encased cotton swab that subjects put in their mouth for 30 s then placed back in a tube for transport and analysis. Subjects drank the ^2H_2O vials three times a day for 2 days to ramp up to a body water enrichment of approximately 0.25 % ^2H_2O. Saliva samples were collected each day to confirm subject adherence to the labeling protocol and to determine ^2H_2O enrichment of body water. They also kept a log of their water use. On the third day, a SF lavage sample was obtained. Specifically, 3 mL of sterile saline was injected into the joint space, the joint was flexed three times to mix with SF, and an aliquot of SF lavage was aspirated out (at least 2 mL). Additionally, a 5 mL sample of blood was obtained into a heparinized tube and processed to plasma. After the SF aspiration, the study was complete for all subjects. Aliquots of the SF lavages, plasma, and salivettes were frozen at −70 °C for transport to KineMed, Inc., (Emeryville, CA). The remainder of the samples was saved for cytokine, bradykinin, and leptin analyses.

Determination of ^2H_2O enrichment in biofluid specimens

Salivettes were defrosted and centrifuged to collect liquid. These saliva samples, along with plasma and SF lavages, were analyzed for ^2H_2O enrichment using high-precision laser spectroscopy [30].

Crude isolation of HA and CS from SF lavage samples was achieved for gas chromatography/mass spectrometry analysis [31]. Aliquots of SF lavages were transferred to microfuge tubes fitted with filter cups with 3-kDa cellulose filters (Pall) and centrifuged for 60 min at 10,000*g* to retain macromolecular material. To isolate HA from the retentate, 50 mM Tris–acetate (pH 8) including 0.1 U chondroitinase ABC (Sigma-Aldrich, St. Louis, MO) and 0.02 % bovine serum albumin (BSA; Sigma) was added to the filter cups, allowed to incubate at 55 °C overnight, and the new filtrate was collected following a second centrifuge cycle (60 min at 5,000*g*). This second filtrate solution, containing the disaccharide products of chondroitinase digestion (dermatan sulfate, CS, and HA disaccharides), was hydrolyzed to by incubation with equal parts 3 N methanolic HCl for 1 h at 20 °C.

Fractional synthesis measurements of HA and CS

Fractional synthesis is defined as the fraction of a given metabolite that was synthesized (new) during labeling period. Following labeling with ^2H_2O, we determined the fractional synthesis of HA using gas chromatography/mass spectrometry (GC/MS) to quantify the proportion of the collected analytes that were labeled with deuterium. HA and CS hydrolysis products were derivatized to pentofluorobenzyl derivatives and acetylated, as described elsewhere [31]. N-acetylglucosamine (glcNAc) and N-acetylgalactosamine (galNAc) were analyzed by GC/MS as representative of HA and CS, respectively. For each derivative, mass isotopomer abundances were determined using negative chemical ionization. GC retention times of all derivatives were established by use of unlabeled standards, and the M0, M1, and M2 mass isotopomers were analyzed by selected ion monitoring, using a model 5973 mass spectrometer attached to a 6890 gas chromatograph (Agilent, Palo Alto, CA). Isotopic enrichment (EM1) in glcNAc or galNAc was then calculated by subtracting the natural abundance background. This enabled us to derive turnover rate constants from the measured EM1, and a mathematical model was used to account for the short labeling time, the time-varying nature of the labeling protocol, and the number of ^2H_2O-exchangeable hydrogens (6 for both glcNAc and galNAC) [31].

Statistical methods

The trial's primary objective was to determine the effects of oral GAGs on pain intensity and WOMAC osteoarthritis index. Primary analyses were conducted by intent to treat using the last observation carried forward technique for missing data, with participants analyzed according to their initial assignment. All tests of hypotheses and reported *P* values were two-sided.

To compare groups, *t* tests were used for continuous demographic or clinical data and Chi-square or Fisher's exact tests for categorical data.

The effects of oral GAGs at 3-month post-randomization were determined by repeated measures analysis of covariance (ANCOVA), using the statistical package SAS 9.1 (SAS Institute, Inc., Cary, NC). The main models included follow-up levels as the dependent variables with group, time, and their interaction as independent variables and age, gender, BMI, and baseline levels as covariates. Separate models were also fitted for change from baseline adjusting for age, gender, and BMI. *P* values for group differences at each time period are reported as they reflect the effect of the interventions at the end of the studied period. *P* values for differences over time were obtained from models with their interaction with the effect of the treatment. Results from these models are reported in the text only. Post hoc correlations between clinical variables and age, gender, BMI, and baseline levels were also obtained.

A sample size of 40 subjects (20 per group) provided 80 % power at the alpha level of 0.05 to detect a mean changeover time that is about equal to the within-group standard deviations for the changeover time in score points for WOMAC and VAS pain scale end points, which were estimated from previous pilot studies in subjects with osteoarthritis (unpublished results).

For the multiple cytokine complex, we used the software that was provided from the company for the analysis.

The bradykinin and leptin were single direct measurement from ELISA, and data were compared with a *t* test using SigmaStat software (Systat Software, Inc., San Jose, CA).

For the $^{2}H_2O$ data, all data are expressed as mean ± standard deviation. To justify the use of parametric statistical tests, we confirmed that data were distributed in a Gaussian manner using both the Kolmogorov–Smirnov and the D'Angostino–Pearson omnibus tests for normality. When either of these tests failed to confirm normality, we used nonparametric statistics instead. The effects of oral GAGs were compared by one-way ANOVA.

Results

Baseline characteristics

Fifty-one patients met clinical inclusion criteria and were randomized to receive either oral preparation or placebo; 40 (78 %) patients completed the study (Fig. 1). Baseline characteristics of the patients included in the intent-to-treat population are summarized in Table 1. At baseline, the study population had a mean age of 60.8 years, mean BMI of 34.6, and mean VAS score of 64.8 mm. All participants had clinical knee OA with an average KL grade of 2.5, symptoms greater than 3 months, and clinical knee effusion. Most of the participants (95 %) had MS. Of the participants, 82.5 % had two or more MS components, 87.5 % were overweight, 72.5 % hypertensive, 75 % dyslipidaemic, and 87.5 % impaired glucose metabolism. Participants receiving oral preparation and placebo were well matched in terms of demographics and disease characteristics with the exception of a statistically significant lower proportion of placebo patients with diabetes ($P = 0.008$).

Table 1 Basal characteristics of patients

	Oralvisc ($n = 21$)	Placebo ($n = 19$)	*P* value
Age (years)	60 (51–75)	62 (45–74)	0.960
Sex (female/male)	9/12	11/8	0.355
BMI (kg/m^2)	35.04 ± 1.02	34.04 ± 1.46	0.860
Metabolic syndrome			
Overweight, *n* (%)	20 (95.2)	15 (78.9)	0.126
Hypertension, *n* (%)	17 (80.9)	12 (63.2)	0.218
Dyslipidaemia, *n* (%)	17 (80.9)	13 (68.4)	0.374
Impaired glucose metabolism, *n* (%)	13 (61.9)	4 (21.0)	0.081
KL score (0/1/2/3/4)	(1, 4, 6, 7, 3)	(0, 2, 6, 7, 4)	0.363
Pain intensity (VAS score)	6.7 ± 0.18	6.2 ± 0.21	0.348
Knee function (WOMAC total)	40.3 ± 2.55	40.5 ± 3.40	0.960

Data expressed as mean ± standard deviation or *n* (%)

BMI body mass index, *KL* Kellgren–Lawrence, *VAS* visual analog scale, *WOMAC* Western Ontario and McMaster Universities Osteoarthritis Index

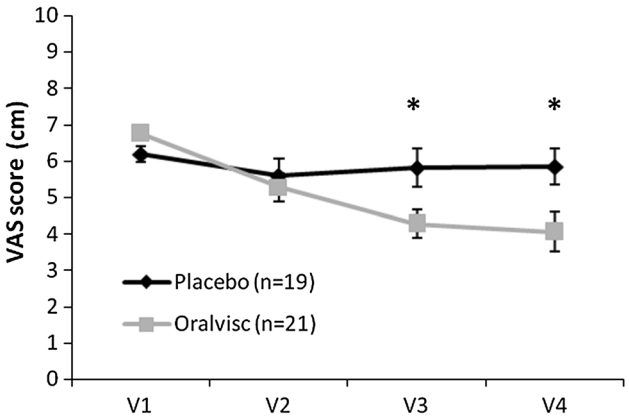

Fig. 2 Visual analog scale (VAS) pain scores at rest (mean ± SE), at baseline (visit 1, *V1*), and during treatment (intermediate visits, *V2*, *V3*, and final visit, *V4*) with Oralvisc and placebo (perprotocol population)

Pain and function efficacy results

The oral preparation was superior to placebo for all three clinical end points, as indicated by a significantly greater mean change from baseline in VAS pain score (Fig. 2). Improvements in VAS pain score were statistically superior ($P < 0.05$) for oral preparation compared with placebo from weeks 8 through 12, while improvements in WOMAC pain subscale and WOMAC function reached statistical significance at 12 weeks (Table 2). There was a positive correlation between BMI and WOMAC function at baseline ($r = 0.033$; $P = 0.0362$). There is a negative correlation between BMI and the evolution of WOMAC function along the experimental period for the placebo group ($r = -0.50$; $P = 0.0309$), but not for the oral preparation group ($r = -0.066$; $P = 0.7773$).

Bradykinin results

Both serum and SF levels of bradykinin were significantly lower in the oral preparation group ($P < 0.05$). The final serum bradykinin levels were significantly lower for oral preparation 1.44 ng (95 % confidence intervals, 1.36, 1.50) versus placebo 1.51 ng (1.34, 1.67) ($P < 0.05$). The final SF decrease in bradykinin was significantly more for oral preparation 0.61 ng (0.28, 0.93) versus placebo −0.29 ng (−0.65, 0.05) ($P < 0.05$). There was a significant decrease in final compared to initial SF bradykinin levels in the oral preparation group ($P < 0.05$) (Table 3). There was no correlation between an individual's serum and SF level of bradykinin or leptin.

Chemokine and cytokine results

There was a significant decrease between the initial and final serum levels in interleukin-1α (IL), IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17α, interferon (IFN), tumor necrosis factor alpha (TNF-α), and granulocyte macrophage colony-stimulating factor (GM-CSF) in the oral GAGs group ($P > 0.05$). The placebo group had significant increases in IL-1α, IL-1β, IL-2, IL-4, IL-6, and TNF-α ($P > 0.05$). There was also a significant decrease between the initial and final SF levels in most chemokines and cytokines in the oral GAGs group ($P > 0.05$), whereas the placebo group had significant increases in IL-10, IFN, TNF-α, and GM-CSF ($P > 0.05$). Overall, the oral preparation had decreased serum and synovial cytokine levels while patients on placebo had increased levels over the same 12 weeks.

Leptin results

There was a significant decrease between the initial and final serum (Table 3) and SF leptin levels in the oral preparation group ($P > 0.05$) as measured by immunoassay. There was also a significant decrease in the oral preparation serum and synovial final leptin levels as compared to placebo. There was no significance in the relationship of BMI or MS scores to initial or 12-week oral preparation usage to leptin levels. However, the oral preparation group lost an average of 0.55 kg compared to a 0.75 kg weight gain in the placebo group over the 12 weeks ($P = 0.07$). There was

Table 2 VAS and WOMAC values

	Oralvisc ($n = 21$)		Placebo ($n = 19$)	
	Baseline	3 months	Baseline	3 months
VAS	6.75 ± 0.18^{a}	$4.06 \pm 0.53^{b*}$	6.18 ± 0.21	5.84 ± 0.50
WOMAC pain	8.81 ± 0.67^{a}	$5.75 \pm 0.90^{b*}$	8.05 ± 0.77	8.16 ± 0.73
WOMAC total	40.29 ± 2.55^{a}	$27.62 \pm 4.38^{b*}$	40.53 ± 3.40	39.58 ± 3.97

Values are mean ± SE. Different superscripts indicate significant differences across time within a group treatment. An asterisk indicates significant differences between groups at specified time point ($P < 0.05$)

VAS visual analog scale, *WOMAC* Western Ontario and McMaster Universities Osteoarthritis Index

Table 3 Bradykinin and leptin concentrations in serum and synovial fluid

	Oralvisc ($n = 21$)		Placebo ($n = 19$)	
	Baseline	3 months	Baseline	3 months
Serum				
Bradykinin (ng/mL)	15.53 ± 0.28^{a}	$14.44 \pm 0.38^{b*}$	15.22 ± 0.28	15.06 ± 0.81
Leptin (ng/mL)	21.72 ± 0.47^{a}	$20.86 \pm 0.55^{b*}$	21.62 ± 0.52	21.38 ± 0.58
Synovial fluid				
Bradykinin (ng/mL)	11.15 ± 0.25^{a}	$10.55 \pm 0.26^{b*}$	11.02 ± 0.81	11.31 ± 0.82
Leptin (ng/mL)	26.25 ± 0.56^{a}	$20.95 \pm 0.52^{b*}$	25.88 ± 0.41^{b}	27.69 ± 0.49^{a}

Values are mean ± SE. Different superscripts indicate significant differences across time within a group treatment. An asterisk indicates significant differences between groups at specified time point ($P < 0.05$)

no mention of nausea or other gastrointestinal complaints from preparation or placebo.

Compliance and individual ^2H_2O enrichment

Analysis of the ^2H_2O enrichment in the patients' body water from saliva and serum samples indicated that all patients were self-administering the ^2H_2O doses according to the instructions. Patients on the drug did not exhibit any differences in ^2H_2O enrichment compared to placebo-treated patients (data not shown). The levels of ^2H_2O enrichment achieved in each patient were variable, increasing as expected during the period of ^2H_2O dosing (reaching 0.22 ± 0.08 % $P \sim 1.5$ days after the first dose) and then exhibiting a slight die-away until the serum sampling time.

SF analysis for ^2H_2O labeling of HA

Synovial fluid (SF) lavages were collected from 20 patients. The volume of processed SF obtained (following lavage) averaged 5.5 ± 3.7 mL. Of this amount, a 100 uL aliquot was processed from each patient for GC/MS to quantify the degree of isotopic enrichment with deuterium. Specifically, synthesis rate of HA was determined from the labeling of new molecules with deuterium. A compartmental model was employed to account for the complex, time-varying labeling regimen.

The rate of HA turnover was 78 ± 42 %/day (i.e., a half-life of 0.9 days) in placebo-treated OA patients ($n = 10$; Fig. 3). With 12 weeks of oral preparation treatment, the mean rate of HA turnover declined by 45 % to 42 ± 24 %/day (a half-life of 1.6 days; ANOVA, $P = 0.046$). The difference was predominately driven by three patients in the placebo group that recorded the maximum turnover rate for this assay at 140 %/day.

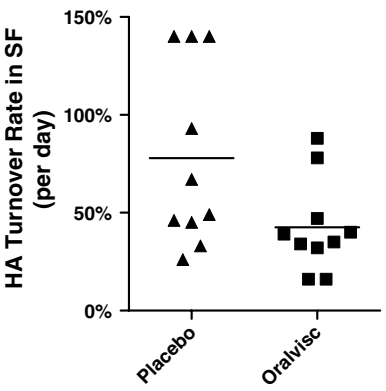

Fig. 3 Turnover rate of hyaluronic acid (HA) in the knee synovial fluid of osteoarthritic patients. *Bar* shows the group mean (ANOVA, $P = 0.046$)

Chondroitin sulfate (CS) molecules in the SF were highly enriched, with the majority of the patients recording the maximum measureable turnover rate for this assay. There was no difference in the CS turnover rate among the two groups, with SF CS turning over at rates of 120 ± 31 % per day and 122 ± 27 % per day in the placebo and treated groups, respectively ($P = 0.9$).

Discussion

It is not surprising that improvement in pain is associated with improvement in function. It has been postulated that intra-articular HA improves joint rheology by increasing SF elasticity and viscosity [32]. There is very little evidence to support this. However, it has been demonstrated that intra-articular HA may improve the symptoms of OA by mitigating synovial cell release of proinflammatory mediators and pain producing neuropeptides [33]. The absorption and distribution of oral HA to connective tissues has been demonstrated [34]. However, the mechanism of action for knee OA remains unclear. Oral HA has been found to affect OA knee pain, particularly in younger persons [35]. The efficacy of chondroitin has been demonstrated with doses between 800 and 1,200 mg/day, not lower. In fact, there are dose–response studies to justify the therapeutic dose of CS. The daily dose of Oralvisc is 80 mg/day (10-fold lower than CS). In addition, 70 % of Oralvisc is pure HA (56 mg/day) and the rest up to 24 mg/day is composed by GAGs. In conclusion, the efficacy could not be attributed to CS, but to the patented composition of HA and GAGs. The oral bioavailability of HA has been previously reported [34]. The mechanism of the effects of oral HA may be due to its effect on intestinal Toll-like receptors [36]. The improvement in pain and function confirms our first hypothesis. The number of placebo responders was unusually low. A pair plot showed only 2 of 19 placebo patients with VAS > 2 pain relief compared to 14 of 21 in the treatment group. We emphasize that our selection criteria were based on the reality of an orthopedic practice, wherein patients are most often seen because of pain which is often associated with an acute knee effusion. All patients in this study presented with a knee effusion, which is not typical of the ACR selection system. Hence, this population was, for the most part, entering a phase of an increased inflammatory response. There are no safety issues in that the product has been commercialized in Europe without any serious adverse event reported.

Patients with OA have elevated levels of inflammatory cytokines and chemokines in their SF [37]. The changes in cytokines and chemokines suggest that the oral preparation group has less inflammation as compared to placebo. Overall, these results point to the oral preparation working

systemically with a decrease in proinflammatory mediators. It would be speculative as to why anti-inflammatory cytokine IL-10 had a significant decrease in the group treated with the preparation. It is known IL-10 has a direct anti-inflammatory, -catabolic and -apoptotic potential of IL-10 in cartilage suggesting a chondroprotective effect of IL-10 in RA and OA, as well as non-RA and non-systemic cartilage disorders [38].

Bradykinin receptors exist in chondrocytes and on stimulation increase IL-1. Bradykinins are known to participate in innate immunity, inflammation, and pain [39]. Hence, evidence of reduction in bradykinin levels is clinically relevant.

Serum concentrations of leptin are increased in obese humans with knee OA [40]. Overweight and obesity have been shown to be major risk factors for OA. Leptin has been shown to be present in the SF of OA patients, and it has been suggested to play a role in cartilage homeostasis [41]. Leptin has been shown to have a role in matrix metalloproteinase-13 (MMP) regulation in cartilage [42]. MMP-13 has been implicated in type II collagen degradation in OA cartilage [43]. Therefore, lowering serum and SF levels of leptin can prove to be beneficial in cartilage homeostasis and turnover. The combined effect of the oral preparation on chemokine, cytokine, bradykinin, leptin serum, and SF levels confirms our third hypothesis.

Our fourth hypothesis was met with detectable changes in CS turnover in that the rapid accumulation of labeled CS in the SF suggests that in OA CS, moieties overwhelmingly arise from recently synthesized matrix molecules, rather than from the degradation of preexisting matrix. There were also effects on HA turnover. It was found that following 12 weeks of oral preparation treatment, the rate of HA turnover in the SF was reduced in osteoarthritic knees, nearly doubling the half-life of HA in the joint fluid. Given the study design, these rates were not directly compared with the rate of HA turnover in normal knees. HA has a defined and essential role in the biomechanics of normal SF (e.g., lubrication and viscosity). Moreover, in end-stage OA, HA concentration in the knee is significantly decreased.

The link between obesity and OA has been widely reviewed [44–47]. OA is now viewed as a process that affects the whole joint, including articular cartilage, subchondral bone, synovial capsule and membrane, and the periarticular (connective and muscular) tissues [1, 48]. Dysregulated production of adipose tissue-secreted inflammatory mediators, hyperlipidemia, and increased systemic oxidative stress are conditions frequently associated with obesity that may also favor joint degeneration [44]. One adipokine in particular, leptin, has been shown to be a critical mediator of obesity-associated OA via synergistic actions with other inflammatory cytokines [45]. To our knowledge, this is the first clinical trial in which it has been demonstrated that an oral intervention induced a clinical OA improvement, which is simultaneous to a normalization of parameters related to obesity, such as hyperleptinemia and systemic upregulation of inflammatory cytokines. Moreover, the regulation of the inflammatory milieu detected both in serum and SF in the patients treated with oral preparation has been associated with a trend toward a normalization of the HA turnover in SF. This partial normalization could suggest a slower OA progression since HA turnover has been associated with disease severity in preclinical models [49, 50].

Conclusion including clinical impact and analyses impact

The overall results suggest that the oral treatment with Oralvisc is safe and effective for the treatment for patients with OA, knee effusion, and obesity. In particular, it reduces pain and has an effect on local and systemic inflammation and on SF turnover.

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