Original article

Testing a novel bioactive marine nutraceutical on osteoarthritis patients

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Abstract. Osteoarthritis (OA) is a slow, chronic joint disease characterized by focal degeneration of articular cartilage and alterations of the chemical and mechanical articular function and also major cause of pain and physical disability. There is clinical evidence that increasing dietary n-3 relative to n-6 may be beneficial in terms of symptom management in humans but not all studies conclude that dietary n-3 PUFA supplementation is of benefit, in the treatment of OA. Our recent studies highlight the effect of a biomarine compound (LD-1227) on MMPs, collagen metabolism and on chondrocyte inflammatory markers. Thus, the aim of the present work was to test such bioactive compound versus a common nutraceutical intervention (glucosamine/chrondroitin sulfate) in knee osteoarthritis patients. The patients population consisted of 60 subjects with a recent diagnosis of knee osteoarthririts of mild-moderate severity. Patients were randomized in a double-blind study comparing LD-1227 (group A) versus a mixture of glucosamine (500 mg), chondroitin sulfate (400 mg) (group B). Patients were allowed their established painkillers on demand. At 4, 9 and 18 weeks patients were evaluated as for: VAS score assessing pain at rest, and during physical exercise, Lequesne index, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale and KOOS scale. Moreover, serum concentrations of IL-6, IL-β, CRP, TNF-sR1 and TNF-sR2 were assessed. As compared to GC treatment, LD-1227 yielded a quicker and higher degree of improvement of the whole clinical indexes and a lower NSAIDs use at the end of the study. LD-1227 brought about also a more significant downregulation of the tested cytokines cascade. Taken overall, these data suggest that LD-1227 has the potential to be included in the nutraceutical armamentarium in the management of OA. (www.actabiomedica.it)

Key words: LD-1227, osteoarthritis, glucosamine, chondroitin sulfate

Introduction

Osteoarthritis (OA) is a common and progressive chronic disease leading to impaired joint function and can result in immobility mostly in elderly people (1). Radiographic evaluation of knee x-ray images taken from the Framingham osteoarthritis study showed alterations in Kellgren/Lawrence Grade > or =2 in 44% of parents (mean age 72 years) and 22% of offspring (mean age 54 years) and, indeed, a strong genetic component is suspected (2). Patients suffering from OA often show a discrepancy between objective findings reported by x-ray or magnetic resonance imaging examinations and patient-reported pain and functional restrictions. The sudden onset of disabling joint pain and articular effusion is one typical clinical feature of decompensation indicating the term activated OA. Although several risk factors like overweight or

the family history of OA are well known, the mechanisms on the cellular level leading to cartilage degeneration are not completely understood in detail. Besides other agents, recent data focus on oxidative and nitrosative stress as one aspect involved in the pathogenesis of OA (3). In the course of the growing knowledge about the relevance of free radicals for cellular ageing, their involvement in degenerative diseases of the joint has come into focus of more detailed investigations. On a cellular and subcellular level, proinflammatory agents such as interleukin 1 (IL-1), and tumor necrosis factor (TNF) α are also overexpressed in chondrocytes and joint stromal cells in OA. Such local inflammatory cytokines and the trafficking of inflammatory cells through OA synovium and back out into the circulation (4), explain the presence of some systemic inflammatory markers at higher than normal levels in the blood of subjects with OA and some of these may correlate with specific OA phenotypes. PUFAs are essential fatty acids and precursors to a number of important factors called eicosanoids, such as prostaglandins, thromboxanes, leukotrienes and resolvins. Few experimental studies have investigated the effects of n-3 PUFAs on OA, although there is clinical evidence that increasing dietary n-3 relative to n-6 may be beneficial in terms of symptom management in humans (5). Not all studies however conclude that dietary n-3 PUFA supplementation is of benefit, in the treatment of OA (6). Our recent studies highlight the effect of a biomarine compound (a sturgeon egg-derived homogenate which has a protein/lipid ratio of 3.6 and the following major class of fatty acids (saturated fatty acids: 23%, monounsaturated fatty acids 33%, polyunsaturated fatty acids 34%, with a median n-6/n-3 fatty acid ratio of 2.7. In particular, the main fatty acids/100g of total fatty acids are as follows: C22:6 5.8, C16:0 15.8, C18:1 33.7 and C18:2 24.4. LD-1227, Caviarlieri, LabDom, Switzerland) on MMPs, collagen metabolism and on chrondrocyte inflammatory markers (7, 8). Thus, the aim of the present work was to test such bioactive compound versus glucosamine/chrondroitin sulfate, a common nutraceutical intervention in osteoarthritis patients (9, 10). In particular, specific end-points of the study were: 1) to test the tolerability of this compound; 2) its efficacy on pain management; 3) its efficacy on inflammatory markers and 4) the potential reduction of anti-inflammatory drugs consumption.

Materials and Methods

The study was carried out in accordance with the principles of the Helsinki Declaration of 1964 and subsequent updates; an informed consent was obtained from all patients. The patients population consisted of 60 subjects (table 1) with a recent diagnosis of knee osteoarthririts of mild-moderate severity according to the criteria of the American College of Rheumatology and different degrees of radiological damage as from Kellgren classification (11) (table 2) whose validity has been recently confirmed (12). Patients with a radiological Kellgren score of I–III were included in the study.

After the use of usual medications had ceased for 7 days, the 0-100 mm visual analog scale (VAS) score assessing pain during the most painful knee movement had to be more than 40 and Lequesne's functional in-

Table 1. Study population characteristics of osteoarthritis patients

Age (years m ± SD)	64.8 ± 8.8
Gender (M/F)	44/31
BMI (kg/m2; m ± SD)	24.5 ± 6.4
Disease duration (years m ± SD)	5.4 ± 6.3
Radiological score	
I	23
II	35
III	17

BMI: body mass index

Table 2. Kellgren-Lawrence grading scale

Grade 0:	normal
Grade 1:	doubtful narrowing of joint space and possible osteophytic lipping
Grade 2:	definite osteophytes, definite narrowing of joint space
Grade 3:	moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
Grade 4:	large osteophytes, marked narrowing or joint space, severe sclerosis and definite deformity or bone contour

dex over 7 points in order to be included in the study. Lequesne index was used to evaluate the efficacy of therapeutic interventions and uses a questionnaire that asks the patient about perceived pain, activities of daily living, the amount of walking. Participants had to be able to walk and give both verbal and written information regarding the study. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale and Knee disability and Osteoarthritis Outcome Score (KOOS) scale (assessing the knee injury and OA outcome score and divided into five subscales: pain, other Symptoms, function in daily living, function in sport and recreation and knee related quality of life) were also used. KOOS score has high test-retest reproducibility with an intra-class correlation coefficient >0.75 (13). Patients were randomized in a double-blind study comparing LD-1227 (group A) versus a mixture of glucosamine (500 mg), chondroitin sulfate (400 mg) (group B). Patients were instructed to continue their established non-pharmacological treatments (physical exercise) and non-steroidal anti-inflammatory drugs (NSAIDs; 150 mg Diclofenac, 20 mg Piroxicam, 750 mg Naproxen), which were to be consumed on demand and noted daily in a diary. However, subjects were advised not to take the rescue medicine at least 3 days before each evaluation.

Furthermore, we advised patients not to utilize corticosteroids or hyaluronic acid infiltrations. Follow up visit were carried out by a blinded rheumatologist at 4, 9 and 18 weeks. The medications and other treatments in use for concomitant illnesses were recorded at the baseline visit and during the study whenever these treatments were modified. Analyses of the above end-points were based upon the time-weighted average change from baseline. Exclusion criteria were an underlying inflammatory arthropathy, hyperuricemia, expectation of surgery in the near future, recent injury in the area affected by OA of the knee, intra-articular corticosteroid injections within the last 3 months, hypersensitivity to NSAIDs, abnormal liver or kidney function tests, history of peptic ulceration and upper GI hemorrhage, uncontrolled hypertension, congestive heart failure, hyperkalemia, pregnancy, lactation and malignant tumors. Patients were instructed not to engage in any sport activity while maintaining a routine physical activity (walking). Systemic side effects as well as gastrointestinal discomfort were also assessed.

Blood tests

Blood was collected in the morning (between 7 and 9 A.M.) via venipuncture after an overnight fast. Serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at-80 C. The serum concentrations of IL-6, IL-1β, hs-CRP, TNF-sR1 and TNF-sR2 were determined by using Quantikine high-sensitivity immunoassay kits (R&D Systems, Minneapolis, MN, USA). These variables were measured at baseline, at 4, 9 and 18 weeks by two-site chemiluminescent enzyme immunometric assay method. All samples were measured in duplicate and the average of the 2 values was used for data analysis. Samples with undetectable concentrations were assigned a value corresponding to the lower limit of detection of the assay (2 pg/ml for IL-6 (27%), 4.0 pg/ml (13%) for both TNFα parameters and 0.01 mg/l (22%) for hs-CRP). Duplicate samples that did not provide a coefficient of variation < 10% were re-analyzed and all values were averaged for data analysis.

Results

Both groups were treated for 18 weeks. Patients were evaluated before and after the therapy. No dropouts occurred during the trial and all subjects in both groups completed the treatment. Both supplements were well tolerated and no allergic reaction were reported. However, 36% (9/25) of patients taking glucosamine/chondroitin complained mild dyspepsia.

The on-demand use of NSAIDs in both groups was significantly lower as compared to untreated subjects ($p<0.05$) and similar between the two different treatments. In particular, Group A showed a rapid and substantial decrease in the intake of anti-inflammatory drugs (72% vs. 23% in Group B throughout the study period). A significant proportion of patients in Group A (80%) didn't take any anti-inflammatory drug after 4 weeks of treatment ($p<0.05$ vs Group B: 43%).

Pain assessment

Figures 1 and 2 show the VAS scores divided into two different parameters: pain at rest and pain while performing routine physical activity. Starting from the 4-week observation patients treated with LD-1227 showed a showed a rapid and significant decline in VAS score ($p<0.05$) as compared to the ones supplemented with GC and to baseline (fig. 1). Such significant difference between different treatment groups remained throughout the study period ($p<0.05$ vs GC treatment, $p<0.01$ vs baseline). Indeed, several patients reported pain reduction, especially during bed rest at night with the improvement of proper sleep (incomplete data obtained from those patients reporting at the entrance into the study a poor quality of sleep and confirmed by applying Armband metabolic holter, Sensormedics, Italy).

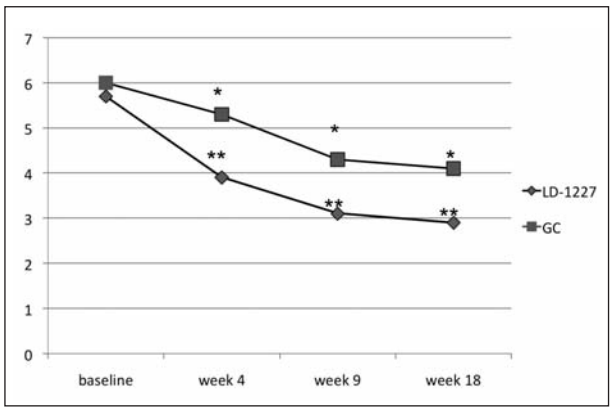

Figure 1. Pain at rest assessed by vas score from baseline through week 18: effect of different nutraceutical implementation treatment
* $p<0.01$ vs baseline; **$p<0.05$ vs group GC

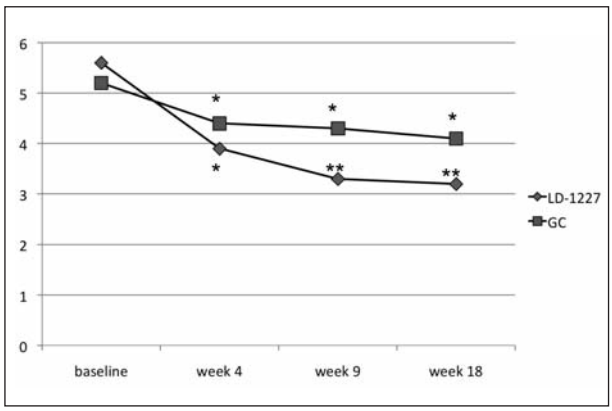

Figure 2. Pain during physical activity assessed by vas score from baseline through week 18: effect of different nutraceutical implementation treatment
* $p<0.01$ vs baseline; **$p<0.05$ vs group GC

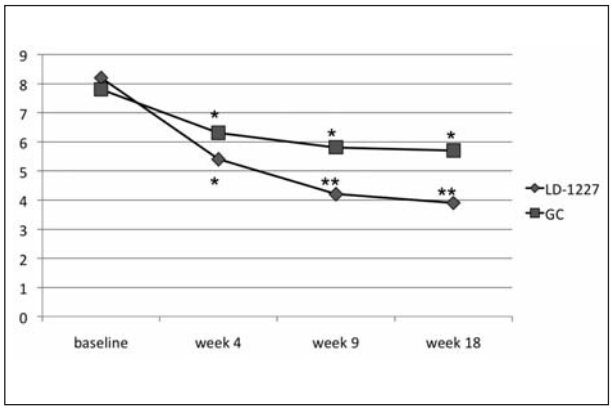

Figure 3. Lequesne index monitored from baseline through week 18: effect of different nutraceutical treatment
* $p<0.01$ vs baseline; **$p<0.05$ vs group GC

As for the assessment of pain during routine physical activity both treatments yielded a significant and comparable improvement at 4-week observation (fig. 2, $p<0.05$ vs baseline and vs untreated subjects). However, at 9- and 18-week observation, patients treated with LD-1227 showed a significantly lower pain intensity as compared to the ones supplemented with GC.

Also the Lequesne index showed an improvement exclusively following the supplementation of both supplement (fig. 3, $p<0.01$ vs baseline) although this appeared, starting from the 4-week observation, to be more significant during LD-1227 treatment ($p<0.05$ vs GC), equalled at 9-week observation, to return to be more significant for LD-1227 at the end of the study ($p<0.05$).

KOOS scores are shown in table 3. KOOS scores improvement for function in recreation was statistically significant only in Group A, as early as at week 4 of treatment. At the end of the study the whole parameters of KOOS scores were statistically better in the group treated with LD-1227 as compared to GC. WOMAC scores are shown in table 4 and confirmed the reductions in pain observed with the KOOS scale. In particular, pain according to the WOMAC score significantly improved only for Group A, as early as at

Table 3. Koos scale evaluation of different nutraceutical treatment of subjects with knee osteoarthririts

	Baseline		4-week		9-week		18-week	
	LD-1227	GC	LD-1227	GC	LD-1227	GC	LD-1227	GC
Pain	44.3	42.7	49.8	48.1	59.7**	52.4	66.4**	58.4*
Function in daily living	52.6	50.4	55.5	54.5	59.8*	57.7*	63.5**	59.3*
Function in recreation	33.3	34.0	37.9**	35.8	39.4**	35.3*	48.4**	38.6*
Other symptoms	53.2	56.1	55.6	57.5	64.2*	62.2*	71.3**	66.5*
Quality of Life	37.6	39.3	44.1	41.7	48.3*	49.3*	56.6**	52.4*

* p<0.01 vs baseline; **p<0.05 vs group GC

Table 4. Womac assessment of patients with knee osteoarthritis treated with two different nutraceuticals

	Baseline		4-week		9-week		18-week	
	LD-1227	GC	LD-1227	GC	LD-1227	GC	LD-1227	GC
Pain	13.5	14.6	8.2**	12.7	4.1**	8.2*	3.5**	6.9*
Stiffness	8.8	10.1	4.1*	5.8*	3.1**	4.8*	2.4**	4.6*
Difficulty in normal daily activity	28.4	31.7	12.4*	15.9*	10.1**	14.9*	8.9**	13.7*
Global score	50.7	56.4	24.7*	34.4*	17.3**	27.9*	14.8**	25.2*

* p<0.01 vs baseline; **p<0.05 vs group GC

weeek 4 (p<0.05 vs GC). Significant improvements in stiffness and difficulty in carrying out normal physical activities were observed by week 4 by both treatments (p<0.01 vs baseline) although LD-1227 achieved significantly better results (p<0.05 vs GC).

Biochemistry

Either routine chemistry, selenium and vitamin D levels were within normal limits (data not shown). Table 5 shows the variation of tested cytokines during the 19-week study period under different nutraceutical treatment. As compared to GC treatment, LD-1227 showed a statistically significant reduction of IL-6, hs-CRP and TNF-sR1 already at 4-week observation (p<0.05). The latter two parameters didn't show any significant modification after GC treatment at 4-week observation. At the end of the study period, all tested variables were more significantly reduced by LD-1227 when compared to GC (p<0.05).

Discussion

Osteoarthritis (OA) is a slow, chronic joint disease characterized by focal degeneration of articular carti-

Table 5. Biochemical monitoring of inflammatory markers in patients with knee osteoarthritis treated with different nutraceutical interventions

	Baseline		4-week		9-week		18-week	
	LD-1227	GC	LD-1227	GC	LD-1227	GC	LD-1227	GC
IL6 (pg/ml)	3.51 ±0.57	3.26 ±0.52	1.88** ±0.45	2.49 ±0.29	1.21** ±0.54	1.96* ±0.47	1.28** ±0.25	1.88* ±0.35
IL-beta (pg/ml)	0.84 ±0.12	0.93 ±0.22	0.51* ±0.21	0.68* ±0.17	0.32** ±0.07	0.51* ±0.13	0.34** ±0.11	0.43* ±0.13
hs-CRP (mg/L)	2.25 ±0.43	2.43 ±0.27	1.23** ±0.34	2.01 ±0.23	0.92** ±0.31	1.89* ±0.23	0.94** ±0.61	1.92* ±0.37
TNF-alpha sR1 (ng/ml)	2.2 ±0.15	1.9 ±0.11	1.3** ±0.08	1.9 ±0.12	1.2* ±0.07	1.4* ±0.06	1.1* ±0.13	1.4* ±0.16
TNF-alpha sR2 (ng/ml)	3.7 ±0.31	3.9 ±0.26	3.4* ±0.21	3.1* ±0.19	2.3** ±0.17	2.9* ±0.22	2.1** ±0.18	3.2* ±0.12

* p<0.01 vs baseline; **p<0.05 vs group GC

lage and alterations of the chemical and mechanical articular function and also major cause of pain and physical disability (1, 14, 15). OA is now thought to involve a complex interaction of biological and pathological processes influenced by a number of factors, including genetic, age, gender, ethnicity, nutritional factor and obesity (2, 16-21). OA places an enormous economic burden on society, which will remain a major health care challenge with an aging population and its management is primarily focused on palliative relief using agents such as NSAIDs. However, such an approach is limited by a narrow therapeutic focus that fails to address the progressive and multimodal nature of OA while not substantially representing an advisable maintenance treatment strategy anyway. Indeed, guidelines for the management of patients with OA of the knee recommend a combination of pharmacologic agents and non-pharmacologic modalities with the goals of relieving pain and improving functional limitation and there is growing interest on the potential role played by nutritional factors in the maintenance of bone and joint health (22, 23). Given that some specific nutrients and vitamins have been reported to be impaired in some cases of OA (24-26), in our study we specifically tested selenium and vitamin D and found them normal, thus avoiding further interpretation biases of the data. Glucosamine and chondroitin sulphate are the most commonly used complementary medicines in Western societies in chronic joint disease as a recent Australian survey has shown (27). Studies have suggested these compounds may have a carryover effect like disease modifying agents and long-term treatment of glucosamine sulfate has been advised to reduce the dependence of NSAIDs usage and delay the disease progression (28, 29). Our findings showed that treatment with LD-1227 proved to act significantly more quickly than GC in pain relief, especially at rest, and to yield the most effective therapeutic action overall at the end of the study period. These observations came not only from the single VAS-scored evaluation of pain but also when analyzing the overall clinical well-being by specific tools such as WOMAC and KOOS which have a widespread consensus as for their applicability and reliability (30-33). Moreover, although the limitation of data do not allow us to draw a significant conclusion, it appeared that subject complaining poor sleep when entering the study, reported a substantial improvement of if as also appeared by a sleep-monitoring device. This incomplete data holds some interest and warrant further studies when considering that quite recently it has been shown that night pain is a meaningful marker of disease severity and may used as a priority indicator for total joint replacement (34). By definition, for the time being there is no cure for osteoarthritis, as it is very challenging to restore the cartilage once it is destroyed. Thus the goals of treatment are to relieve pain, maintain or improve joint mobility, increase the strength of the joints and minimize the disabling effects of the disease. This has understandably spurred a large use and self-medication with complementary drugs (35) mostly of them tentatively aimed to curb the inflammatory process which is invariably associated with this condition (4, 36, 37). Indeed, while the biological onset of OA is not fully understood, an increasing evidence suggests that the progression of cartilage degradation is mediated mostly by proinflammatory cytokines, in particular interleukin 1-beta (IL-1β) and tumor necrosis factor alpha (TNFα) (38, 39). These cytokines contribute to tissue destruction by disrupting the balance of the catabolic and anabolic activities of chondrocytes, the major cell type of cartilage tissue. In the extracellular matrix growth factor and cytokines are present in an inactive form but they can be activated by proteolytic enzymes. Thus, the inhibition of such factors, particularly IL-1β and TNFα, could be a viable therapy for slowing down the progression of OA and disease modification OA drugs, have become increasingly promising therapies (40). At the same time, there is an emerging interest from the medical community towards effective nutraceutical implementation of the therapeutic management of OA aiming to achieve a better maintenance control of the underlying inflammatory changes for a tentative rebalancing of the catabolic/anabolic processes in the cartilage (41). In view of this approach, a Polish group has recently reviewed the potential beneficial anti-inflammatory effect EPA/DHA mixtures on OA management (42). Indeed, it has been clearly demonstrated that OA and the subjective severity of OA pain are associated with low level systemic inflammation (43) and a well-conducted 5-year prospective studies has recently confirmed the robust association between inflammatory markers and change in knee

pain where changes in hs-CRP and TNF-α were related with fluctuation in knee pain at night and when sitting/lying and with change in total knee pain, respectively (44). This further highlight the potential relevance of the present study were we showed at a biochemical the effective anti-inflammatory action of LD-1227 as compared to baseline and, most importantly, to GC supplement. Such key inflammatory markers control remained throughout the study period and one can suggest that it has to be advocated for the better clinical results obtained by this marine bioactive compound. In a most recent work, we have shown that this sturgeon-derived compound could effectively inhibit IL-1β-induced proliferation and inflammatory reactions via inhibited activation of the transcription factor NF-κB pathway in human chondrocytes derived from OA patients (8). Moreover, recent work from our group has shown that, at least some effects of the present sturgeon extract cannot be simply reconciled with its content of EPA/DHA (7, 45). Indeed, this compound contains collagen elastin, protein and a rich array of other smaller unsaturated fatty acids, and structural phospholipids to be fully defined as yet which may exert a synergistic action on multiple mechanisms of the inflammatory cascade.

Taken overall, these data suggest that LD-1227 has the potential to be included in the nutraceutical armamentarium in the management of OA, being devoid of side effects or known drug interaction and amenable to long-term use.

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