Research Article

Ameliorating Effects of D-003, a Mixture of Sugarcane Wax Acids, on Osteoarthritic Symptoms

Roberto Puente[1], José Illnait[1], Rosa Mas[2], Daisy Carbajal[2], Sarahí Mendoza*[2], Julio Fernández[2], Meilis Mesa[1], Rafael Gámez[2], Pablo Reyes[3], Dalmer Ruiz[3]
[1]Surgical Medical Research Centre, Havana, Cuba.
[2]Centre of Natural Products, National Centre for Scientific Research, Havana, Cuba.
[3]Software and Database Group, National Centre for Scientific Research, Havana, Cuba.
***Corresponding author's E-mail:** clinica@enet.cu

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ABSTRACT

Non-steroidal anti-inflammatory drugs relief osteoarthritis (OA) symptoms but produce adverse effects (AE) that support the search for safer treatments. D-003, a mixture of sugarcane wax acids, has been effective in formaldehyde and monoiodoacetate-induced OA models in rats. The effects of D-003 in subjects with OA symptoms, however, had not been investigated yet. To investigate the effects of D-003 (10 – 20 mg/day) on OA symptoms. Patients with OA symptoms were double-blindly randomized to D-003 (10 mg) or placebo for 6 weeks. Symptoms were assessed by the Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) and the visual analogy scale (VAS) scores. Patients without symptom improvement at week 3 were titrated to double dose. The primary outcome was the total WOMAC score. WOMAC pain, joint stiffness and physical function scores, VAS score and use of rescue medications were secondary outcomes. Sixty (60) patients (mean age: 69 years) were eligible for randomization. At study completion D-003 reduced ($p<0.00001$) total (54.8%), pain (55.1%), joint stiffness (62.4%) and physical function (54.2%) WOMAC scores, and VAS score ($p<0.05$, 49.1%) versus placebo. These decreases, significant from the first week, were enhanced throughout the study. Dose titration and use of rescue medication among D-003-treated subjects (5/30 and 4/30, respectively) were less frequent ($p<0.01$) than in placebo (22/30 and 16/30, respectively). Treatment was well tolerated. There were four study withdrawals, three (2 placebo, 1 D-003) due to AE. The present data indicate that D-003 treatment (10 – 20 mg/day) for 6 weeks ameliorated osteoarthritic symptoms and was well tolerated.

Keywords: Anti-inflammatory, D-003, Osteoarthritis, Sugarcane wax acids, VAS score, WOMAC score.

INTRODUCTION

Osteoarthritis (OA), a painful and disabling degenerative joint disease is the most common musculoskeletal disorder and one of the most prevalent chronic diseases affecting the elderly worldwide. Although the progressive destruction of joint cartilage is the most prominent feature of OA, currently OA is considered as a global disease that involves synovial membranes, subchondral bone, and periarticular soft tissues.[1-3]

Non-pharmacological interventions are the cornerstone of OA management.[4] Nevertheless, despite non-steroidal anti-inflammatory drugs (NSAIDs), which act by inhibiting the enzyme cyclo-oxygenase (COX) activity, and analgesics, like paracetamol, do not solve the underlying pathological processes involved in OA, current guidelines support their use of for symptom relief in OA, in addition to non-pharmacological approaches.[5,6] Incidentally, antiresorptive drugs, like biphosphonates and estrogens have been shown some benefits in OA since they reduce subchondral bone lesions in elderly women with knee OA, as compared to untreated women.[7] It is opportune to remark that despite the evidences that increased oxidative stress and reduced endogenous antioxidant defenses seem to contribute to OA pathogenesis,[8, 9] the use of antioxidants is not included within the guidelines' pharmacological armamentarium to prevent/treat this disease.[5, 6]

Keeping in mind this background and the potential gastro toxicity of non selective NSAIDs, the cardiovascular adverse effects of COX-2 inhibitors and the hepatotoxicity of paracetamol,[10-13] the quest for safer treatments is justified.

Oral administration of D-003, a mixture of high molecular weight fatty acids purified from sugar cane wax whose major component is octacosanoic acid,[14] has shown to be effective for lowering cartilage injury and associated inflammation in formaldehyde and monoiodoacetate-(MIA)-induced OA in rats,[15, 16] and to reduce bone loss and bone resorption in experimental models of osteoporosis.[17-21] Also, D-003 (10 mg/day) given for 6 months was able to reduce the urinary excretion of deoxipirridoline (DPD)/creatinine), a marker of bone resorption, to postmenopausal women with reduced bone mineral density (BMD)[22] and given for 3 years increased spine BMD values in this population.[23]

In light of this background, the present study was conducted to investigate the effects of D-003 (10 to 20 mg/day) on OA symptoms.

PATIENTS AND METHODS

Study design

This randomized, double-blind, placebo-controlled study was approved by the Institutional Ethics Committee of the Surgical Research Centre (Havana, Cuba) and was

conducted following the ethical standards of the Declaration of Helsinki. The study was registered on Cuban Public Register of Clinical Trial (RPCEC00000170). The subjects who were able to provide written informed consent at enrolment were included in the study.

Eligible patients were randomly assigned by a computer-generated schedule to D-003 (5 mg) or placebo tablets, so that two tablets should be taken once a day with the breakfast for 6 weeks. Thereafter, subjects attended to visits every week. Physical examinations and symptom assessment were done at each visit. Treatment compliance, control of rescue analgesic medications and adverse experiences (AE) were controlled weekly, whereas laboratory examinations were done at baseline and after 3 and 6 weeks on treatment.

Study participants

Women and men (20 - 80 years old), with a prior diagnosis of knee, hip, hands/fingers or mixed OA supported by clinical and radiological criteria, were recruited. Participants were required to have a diagnosis of functional class I, II or III (mild to moderate) according to the American College of Rheumatology Criteria (ACRC) criteria [24, 25] and the Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) ≥ 30.[26-29]

Subjects were excluded from the trial if suffered other forms of arthritis, underwent arthroscopy within the past year, any joint replacement, received intra-articular steroids injection within the prior 3 months, or if they had uncontrolled hypertension (diastolic pressure ≥ 120 mm Hg) or diabetes (fasting glucose > 7 mmol/L), active liver or renal disease, malignancies, any other serious illnesses, hospitalizations during the previous 6 months previous or the following lab values: alanine -ALT- and/or aspartate – AST-amino tranferase >45 U/L, creatinine > 130 µmol/L. Also, pregnant and nursing women, as those not taking adequate contraceptive measures were excluded.

Premature dropouts included unwillingness to follow-up, experiencing AE that support such a decision and protocol violations (failure of treatment intake ≥5 days).

Treatment

Study treatments were manufactured in Laboratories MedSol (Havana, Cuba). D-003 content was assessed by using a gas chromatography method.[30] Placebo tablets had similar composition to those of D-003, except the active ingredient that was replaced by lactose. Placebo and D-003 tablets were indistinguishable. Treatments were packaged in identical PVC-aluminium sealed burbles (blisters).

At visit 2, D-003 or placebo tablets were given to study subjects. Two tablets should be taken once daily with the breakfast for 6 weeks, but patients without symptom improvement at week 3 should take another two tablets with the evening meal. Then, D-003-treated patients received doses from 10 to 20 mg/day.

The randomisation code was computer-generated with a fixed, not stratified randomisation method, using balance blocks and allocation ratio of 1:1. The starting dose of D-003 (10 mg/day) selected was that used in studies conducted in postmenopausal women with low BMD. [22, 23]

The entire code was kept confidential at the generating place. Sealed individual coded envelopes, which should be opened prematurely in case of a serious adverse event (SAE), were kept at this site and at that of the Principal Investigator. This premature opening, however, did not occur in the trial since no SAE were experienced.

Treatment compliance was controlled by a count of remainder tablets at each visit and interviews to the subjects. Non-used tablets were recovered at the end of the study. Compliance was considered good if subjects consumed at least 85% of the tablets scheduled from the previous visit.

Subjects were not allowed to consume NSAIDs, steroids, chondroitin, glucosamine, omega-3, omega-6 or calcium supplements, or any agent that may affect the study outcomes, with the exception of the rescue medications to treat persistent pain: acetaminophen (maximum 2 g/day) or metamizole (maximum 600 mg/day). All patients were instructed to keep a diary of their consumption of rescue medications and report them at their next scheduled visit. The number of consumed rescue medication tablets was recorded at each visit.

Outcome Measures

The primary end-point was to obtain a significant decrease of the total WOMAC index[26-29] (Table 1) of at least 30% as compared to placebo. The WOMAC OA index is a validated disease-specific self-administered health status measure that is widely accepted as reflective of OA severity, which supports its use to investigate the effect of any intervention on OA treatment.[26-29]

At each visit, subjects completed the WOMAC questionnaire, which consists of three sections: one evaluates pain intensity (5 questions), other assesses joint stiffness (2 questions), and the third one the physical function (17 questions). Individual responses were scored as follows: 0 (none), 1 (slight), 2 (moderate), 3 (severe) and 4 (extreme). The total score ranges from 0 (the best) to 96 (the worst).

Reductions in WOMAC scores of pain, stiffness and physical functions and in the Visual analogy scale (VAS) score (specific for pain)[31] were secondary efficacy variables. The VAS-visual analogue scale score used a 100 mm linear measure of pain status with 0 as no pain and 100 as the worst experienced pain. Patients marked on the linear scale the relevant amount of pain they were experiencing, and the value was noted. In order to minimize any bias, patients answered to the questionnaires in the doctor's office before their examination.

Decrease in the use of rescue medications versus placebo was another secondary outcome. The amount of rescue medication was assessed in terms of total use at study completion.

Finally, the patient's perception of treatment efficacy on symptom relief was a collateral outcome. Responses were classified as very good (complete symptom relief), good (partial, but relevant symptom relief), fair (modest improvement) or nil (symptoms unchanged). To be effective from this perspective, responses rated as very good or good in the treated group should be ≥70% and significantly greater than in placebo.

Table 1: Modified WOMAC Questionnaire

WOMAC pain assessment at	WOMAC Physical function assessment (difficulty for)	
Walking Stair climbing Night Rest Weight bearing	Descending stairs Ascending stairs Rising from sitting Standing Bending to the floor Walking on flat Getting in/out of a car Going shopping Putting on socks	Rising from bed Taking off socks Lying in bed Getting in/out of bath Sitting Getting on/off toilet Heavy domestic duties Light domestic duties
WOMAC Stiffness assessment		
In morning Occurring during the day		

Responses of subjects corresponded to the following score: 0 = none; 1 = slight; 2 = moderate; 3 = severe; 4 = extreme

Table 2: Baseline characteristics of study population

	D-003 (n = 30)		Placebo (n = 30)		Total (n= 60)	
Age (years) (X±DE)	68 ± 8		70 ± 8		69 ± 8	
Body mass index (kg/m^2) (Mean ± SD)	27.4 ± 4.4		26.9 ± 4.3		27.2 ± 4.3	
Total WOMAC scores (Mean ± SD)	43.7 ± 8.8		42.4 ± 8.3		43.0 ± 8.5	
Degree of OA according to ACRC						
I	0		0		0	
II	9		12		21	
III	21		18		39	
OA site						
Knee	2		3		5	
Hip	1		0		1	
Mixed (hip, knee and hand)	27		27		54	
Gender	**n**	**%**	**n**	**%**	**n**	**%**
Women	22	73.3	22	73.3	44	73.3
Men	8	26.7	8	26.7	16	26.7
Main comorbidities						
Hypertension	26	86.7	25	83.3	51	85.0
Hypercholesterolemia	19	63.3	16	53.3	35	58.3
Overweight (kg/m^2 ≥ 25, < 30)	12	40.0	14	46.7	26	43.3
Diabetes mellitus	8	26.7	8	26.7	16	26.7
Obesity (kg/m^2 ≥ 30)	8	26.7	5	16.7	13	21.7
Coronary heart disease (CHD)	1	3.3	2	6.7	3	5.0
Thyroid dysfunction	1	3.3	2	6.7	3	5.0
Lifestyle factors						
Sedentary life	15	50.0	15	50.0	30	50.0
Smoking	4	13.3	4	13.3	8	13.3
Concomitant therapy						
Consumption of at least one concomitant drug	27	90.0	27	90.0	54	90.0
Angiotensin converting enzyme inhibitors	17	56.7	16	53.3	33	55.0
Diuretics	13	43.3	12	40.0	25	41.7
Cholesterol-lowering drugs	7	23.3	7	23.3	14	23.3
Oral hypoglycemic drugs	7	23.3	7	23.3	14	23.3
β-blockers	6	20.0	3	10.0	9	15.0
Antiplatelet drugs	3	10.0	4	13.3	7	11.7

SD standard deviation, WOMAC Western Ontario and McMaster Individual Osteoarthritis Index**,** OA osteoarthritis, ACRC American College Rheumatology Criteria; [a] The table includes only those consumed by >5 subjects; No significant between group differences were found (t test for independent samples, Fisher Exact Probability test)

Safety and tolerability assessment

Effects on vital signs (body weight, pulse rate, blood diastolic and systolic pressure), and blood indicators (erythrocyte sedimentation rate, ALT, AST, serum fasting glucose and creatinine) were assessed. Blood biochemical safety indicators were measured with enzymatic methods by using reagent kits (Roche, Switzerland) and performed in the Hitachi 709 autoanalyser (Tokyo, Japan). Red blood cell sedimentation rate was assessed by conventional method. All determinations were done at the laboratory of the Surgical and Medical Research Centre (Havana, Cuba). Quality control of the methods was run.

AE was considered as any undesirable event that occurred to a subject, disregarding the cause, whenever they newly appeared during the trial. Subjects were queried for any AE between study visits, and such information was recorded in the case record forms, including AE characteristics, duration, treatments adopted and further responses. AE were classified as mild, moderate or SAE: mild AE were those easily tolerated that not required suspension of study medications and/or specific treatment, moderate those that caused discomfort enough for required stopping therapy and/or specific treatment, and SAE those disabling events that leaded to hospitalisation and/or deaths. AE that happened within 30 days of consuming the last doses, monitored by direct contact with the subjects, were included in this analysis. The causality between AE and treatments was classified by using the Naranjo algorithm. [32]

Table 3: Changes in the total Western Ontario and McMaster Individual Osteoarthritis Index (WOMAC) scores

Week	WOMAC Index scores §§	
	D-003	Placebo
0 (baseline)	43.7 ± 8.8	42.4 ± 8.3
1	31.2 ± 7.4***+	37.5 ± 10.0
2	24.9 ± 10.1***+	32.9 ± 10.3**
3	22.8 ± 11.0***++	35.6 ± 9.3*
4	18.9 ± 9.7***++	32.7 ± 10.5**
5	15.3 ± 10.2***++	34.2 ± 7.5**
6	12.0 ± 10.0***++	34.9 ± 6.8**
% change	72.5	17.7

Values are means ± SD; §§ Divided into three domains: pain, stiffness and physical function. Each domain has several items and each one is graded in a scale of 0 (none) to 4 (extreme), the lowest being the better, the highest the worst. There were a total of 24 items in the total WOMAC score; *p<0.001, **p<0.0001, ***p < 0.00001. Comparisons versus baseline (t test for dependent samples) (Bonferroni adjustment) +p<0.01; ++p<0.00001 (ANOVA)

Statistical Analysis

Data were analysed as per the intention to treat (ITT) approach, which means that data of all randomized subjects were included in all analyses. The sample size estimation assumed that the final D-003-induced reduction of total WOMAC score from baseline should be ≥30% as compared to placebo. Then, 30 subjects per treatment arm would be enough to detect such difference with 80% power and α = 0.05. Assuming a permissible dropout rate of 10%, 66 subjects were enrolled.

Within group differences reflecting changes over time for the same subject (within group comparisons) were assessed for significance with the paired Student's t-test, with Bonferroni adjustment for multiple comparisons. [34] Between group differences were assessed with planned comparisons of one way analysis of variance (ANOVA). Categorical variables were compared with the Fisher Exact Probability test. All statistical tests were 2-tailed. Statistical significance was set at $p < 0.05$. Values are presented as mean ± standard deviation. The following software was used for the comparisons: Statistics software for Windows (USA) and MS Excel.

RESULTS

Baseline characteristics

In total, 60 of 66 enrolled subjects were randomized to active treatment and received study medications immediately after randomization. Six (6) participants were not randomized because of suffering of rheumatoid arthritis (1 subject), and because of serum glucose (3), creatinine (1) and transaminase values above the exclusion criteria (1).

Four (4) subjects were dropped out form this study (2 from placebo, 2 from D-003 group), 1 of them (D-003-treated) due to a protocol violation (failure to take the treatment for 16 days), 2 due to AE: 2 patients (1 of each group) who experienced ankle sprains and other with persistent neck pain and inflammation, not present at baseline. Finally, 56 subjects completed the study.

Baseline characteristics of the two groups were well balanced, so that treatment random allocation was effective (Table 2). Out of 60 participants, the percentage of women (44, 73.3%) was greater than that of men (16, 26.7%). Thirty-nine subjects (65%) were above the normal weight (26 overweight, 13 obese). Comorbid conditions like hypertension (85%) and hypercholesterolemia (58.3%) were very frequent (≥40%) among study participants, and the same was true for sedentary life (50%). Concomitant therapy was also high (54/60, 90%).

Efficacy analysis

Treatment compliance was very good and similar in both groups. The frequency of dose titration among D-003-treated subjects (5/30) was lower (p<0.0001) than in placebo group.

At randomization the total WOMAC scores (mean ± SD) were comparable in D-003 (43.7 ± 8.8) and placebo (42.4 ± 8.3) groups (Table 3). After 1 week of treatment D-003 reduced total WOMAC score as compared to baseline (p <

0.00001) and placebo (p < 0.01) (28.6% decrease versus baseline, 17.0 % versus placebo). The treatment effect did not wear off, but was enhanced throughout the study, so that at the end of the trial D-003 reduced the total WOMAC score to 12.0 ± 10.0 (72.5% decrease versus baseline, 54.9% versus placebo) (p <0.00001 versus baseline and placebo).

From the first week of treatment, D-003 lowered significantly WOMAC pain (p <0.00001 versus baseline; p<0.05 versus placebo) and stiffness scores (p <0.00001 versus baseline; p<0.01 versus placebo), and such effect increased over the trial, so that at week 6 pain score lowered significantly (p <0.00001) the pain score (73.2% versus baseline, 55.0% versus placebo), and the stiffness score (88.9% versus baseline, 62.4% versus placebo (p <0.00001 for all comparisons) (Table 4). WOMAC physical function exhibited a similar reduction with D-003: lowered significantly after 1 week of treatment (p <0.00001 baseline; p<0.05 versus placebo) and such effect was enhanced throughout the trial. At study completion physical function score had been reduced by 70.7% and 54.2% as compared to baseline and placebo, respectively.

Table 4: Changes in pain, stiffness and physical function WOMAC scores by treatment group

Week	Pain score §		Stiffness score §		Physical function §	
	D-003	Placebo	D-003	Placebo	D-003	Placebo
0 (baseline)	9.7± 2.4	9.9 ± 2.4	3.6 ± 1.9	3.4 ± 1.0	30.4 ± 6.6	29.1 ± 6.7
1	7.1 ± 2.1^{****+}	8.6 ± 3.4	2.0 ± 1.3^{****++}	2.8 ± 1.1	22.2 ± 5.7^{****+}	26.0 ± 7.2
2	5.6 ± 2.2^{****+}	7.0 ± 3.1****	1.3 ± 1.1^{****++}	2.4 ± 1.2*	18.0 ± 7.7^{****++}	23.5 ± 7.1*
3	5.0 ± 2.3$^{****+++}$	7.9 ± 2.2*	1.3 ± 1.3$^{****+++}$	2.7 ± 1.2	16.5 ± 8.3$^{****+++}$	25.0 ± 6.7*
4	4.4 ± 2.2$^{****+++}$	7.7 ± 3.1*	0.8 ± 1.0$^{****++++}$	2.4 ± 1.3*	13.7 ± 7.2$^{****+++}$	22.7 ± 7.4*
5	3.5 ± 2.0$^{****+++}$	7.9 ± 2.4*	0.7 ± 1.0$^{****++++}$	2.4 ± 1.2*	11.1 ± 7.6$^{****++++}$	24.0 ± 5.3**
6	2.6 ± 2.1$^{****++++}$	8.1 ± 2.1*	0.4 ± 1.0$^{****++++}$	2.5 ± 1.2*	8.9 ± 7.4$^{****++++}$	24.3 ± 4.9*
% change	73.2	18.1	88.9	26.5	70.7	16.5

Values are means ± Standard Deviation; § Measured on the following scale (0-4, where 0 = none, 1= slight, 2 = moderate, 3 = severe, 4 = extreme). The lowest the better, the highest the worst; *p < 0.0083, **p < 0.0001, ****p < 0.00001. Comparisons versus baseline (t test for dependent samples) (Bonferroni adjustment); $^{+}$p < 0.05; $^{++}$p < 0.01; $^{+++}$p < 0.0001; $^{++++}$p < 0.00001. Comparisons versus placebo (ANOVA test)

Table 5 lists the effect on the mean VAS score. Baseline values were similar in both groups at randomization: 74.7 ± 18.1 in D-003 group, 73.0 ± 18.4 in placebo. At week 1 D-003 reduced significantly VAS score as compared to baseline (p <0.00001), not versus placebo. The effect was accentuated over the study, so that the final reductions were 62.5% and 49.1 % compared to baseline (p< 0.0001) and placebo (p<0.005), respectively.

There were more D-003-treated (27/30, 90%) than placebo (5/30, 16.7%) subjects qualifying treatment efficacy as very good or good (p <0.0001 versus placebo). On its side, 25/30 (83.3%) placebo and only 3 D-003-treated subjects (10%) found the efficacy not good or fair (p <0.0001 versus placebo).

The consumption of rescue medication (acetaminophen or metamizole) in D-003 group (4/30) was lower (p <0.01) than in placebo (16/30).

Safety and tolerability

Treatment was well tolerated. There were four study withdrawals, three due to AE: two ankle sprains that occurred in one subject of each group, and a crisis of neck pain and swollen in a placebo subject. Physical indicators (weight, heart beats, arterial pressure) did not change significantly during the study (data not shown for simplicity). With the exception of serum total cholesterol, which lowered significantly with D-003, the other blood indicators did not change significantly during the study (Table 6), and individual values remained within normal ranges.

Table 5: Changes in VAS scores§

Week	VAS scores §	
	D-003	Placebo
0 (baseline)	74.7 ± 18.1	73.0 ± 18.4
1	62.3 ± 16.6***	69.0 ± 21.1
2	56.0 ± 19.4***	63.0 ± 21.2*
3	51.2 ± 19.6***	63.3 ± 18.1*
4	44.7 ± 21.7***	62.7 ± 18.7**
5	39.5 ± 21.7***	61.7 ± 19.0*
6	28.0 ± 24.2^{***+}	63.2 ± 16.6*
% change	62.5	13.4

Values are means ± standard deviation; § Measured on a 100 mm scale of 0 to 100, where 0 = no pain and 100 was the worst possible pain; *p < 0.0083, **p < 0.0001, ***p < 0.00001, comparisons versus baseline (t test for dependent samples) (Bonferroni adjustment); $^{+}$p < 0.05. Comparisons versus placebo (ANOVA test)

Six participants reported some AE during the study. One (1) D-003-treated subject had an ankle sprain, and 5 subjects from the placebo group experienced an ankle sprain, neck pain and swollen, headache, dizziness,

tonsillitis and leg cramps, respectively, without significant differences between both groups. With the exception of the two ankle sprains (1 occurred in each group) and the neck pain and swollen, which were moderate, the other AE were mild.

Table 6: Effects on laboratory indicators

Treatment	Baseline	week 3	week 6
Total cholesterol (mmol/L)			
D-003	5.58 ± 1.42	5.18 ± 1.12**	5.06 ± 0.85**+
Placebo	5.64 ± 1.19	5.44 ± 0.92	5.60 ± 0.96
Triglycerides (mmol/L)			
D-003	1.51 ± 0.59	1.57 ± 0.64	1.54 ± 0.47
Placebo	1.45 ± 0.75	1.52 ± 0.63	1.57 ± 0.73
AST (U/L)			
D-003	28.67 ± 7.46	26.93 ± 9.63	26.77 ± 6.11
Placebo	27.37 ± 6.99	25.90 ± 11.28	25.00 ± 5.95
ALT (U/L)			
D-003	24.13 ± 9.28	22.17 ± 9.18	22.83 ± 6.41
Placebo	22.00 ± 5.77	22.70 ± 8.89	21.00 ± 6.52
Glucose (mmol/L)			
D-003	5.18 ± 0.81	5.17 ± 0.99	4.99 ± 0.84
Placebo	5.05 ± 0.66	4.99 ± 1.04	4.76 ± 0.84
Creatinine (μmol/L)			
D-003	87.50 ± 20.46	84.17 ± 19.90	85.87 ± 17.40
Placebo	92.67 ± 18.30	89.37 ± 20.61	87.47 ± 20.44
Red blood cells sedimentation rate (mm)			
D-003	17.60 ± 9.00	17.00 ± 7.45	17.07 ± 7.81
Placebo	14.07 ± 7.76	14.83 ± 6.82	14.43 ± 6.57

Values are means ± standard deviation; ** $p < 0.01$, comparisons versus baseline (t test for dependent samples) (Bonferroni adjustment); $^{+}p < 0.05$, comparisons versus placebo (ANOVA test)

DISCUSSION

This study demonstrates, for the first time, that D-003 was able to ameliorate OA symptoms in subjects with mild to moderate OA. Oral intake of D-003 (10 – 20 mg/day) for 6 weeks significantly improved pain, stiffness, physical function and total WOMAC scores, and VAS score for pain as compared to placebo group. The treatment effect, observed from week 1, did not wear off, but was enhanced throughout the study. The benefits were perceived by study participants as supports efficacy self-evaluation.

Both groups were homogeneous at baseline, which indicates that randomization was adequate and that the effects here shown should be attributable to D-003 and not to a lack of baseline homogeneity between the groups. The mean age of study subjects (69 years) is consistent with that expected for OA, a disease predominant in older people. [1-3] In turn, women (73.3%) outnumbered men (26.7%), consistent with a higher prevalence of OA in post-menopausal women,[33,34] a condition present in 41 of the 44 randomized female participants (93.2%). The mean body mass index (BMI) ratio of randomized subjects (27.4 and 26.9 in D-003 and placebo groups, respectively) was above the overweight category, which also agrees with the characteristic features of OA epidemiology.[35]

The high frequency (≥20%) of hypertension (85%), overweight plus obesity (65.1%), hypercholesterolemia (58.3%), sedentary life (50.0%), and diabetes (26.7%) among study subjects, not only reflects the occurrence of concomitant coronary risk factors, common in Cuban subjects of this age,[36] but agrees with reports of co-morbid conditions in middle-aged and older subjects with OA.[37]

D-003 was able to lower significantly total (primary outcome) and subset (secondary outcomes) WOMAC scores from the first week of treatment, and such effects were enhanced thereafter. At study completion D-003 reduced the total WOMAC score and the pain, stiffness, and physical WOMAC scores by 54.9%, 55.0%, 62.4% and 54.2%, respectively, as compared to placebo. The significant reduction of VAS score exhibited a similar pattern, significant from the first week and accentuated over the trial, with a final decrease of 49.1 % as compared

to placebo. Despite the differences in WOMAC and VAS scales, the magnitude of the D-003-induced pain reduction versus placebo evaluated with both tools was quite similar (55.0 % and 49.1% with WOMAC pain and VAS scores, respectively).

It should be noted that placebo group also exhibited decreases of pain, stiffness, physical function and total WOMAC scores (18.2%, 26.5%, 16.5% and 17.7%, respectively and VAS score (13.4%) versus baseline. This modest improvement with placebo should not be surprising, as it has been reported in placebo-controlled studies in subjects with OA.[38] Some factors that may contribute to this results include the facts that participants were advised to remain physically active during the treatment, emphasising for walking 30 minutes every day; and 16/30, 53.3% of placebo subjects used rescue medications over the trial. This modest placebo effect does not limit the demonstration of the efficacy of D-003 for ameliorating OA symptoms, since the significance and magnitude of the reduction of WOMAC and VAS scores and in the use of rescue medication versus placebo support that efficacy results may be attributable to D-003. This appreciation matches well with the fact that 27 D-003-subjects (90.0%) perceived the efficacy as very good (16) or good (11) efficacy, while only 5 (16.7%) placebo referred a good efficacy. Also, although it was not a study outcome, the significant difference of dose titration in both groups (5 D-003, 22 placebo) indirectly supports that D-003 treatment was effective.

The mechanisms by which D-003 may alleviate OA symptoms were beyond the objectives of this study. Nevertheless, experimental models have demonstrated that D-003 effectively reduced all the inflammatory component, cartilage damage and abnormal bone remodeling in the joints of rat with MIA-induced OA, [16] which indicates that multiple, rather a single mechanism, may play a role in the efficacy of D-003 in such a model, and also in the amelioration of OA symptoms here seen. Indeed, the antioxidant and antiresorptive effects of D-003 may have contributed partially to efficacy of D-003 on OA models,[16, 17] and to the present results.

Firstly, lipid peroxidation has been reported to be increased and antioxidant defenses to be reduced in subjects with OA, whereas antioxidants may reduce the incidence and progression of OA. [39] Then, we cannot discard that the antioxidant effects of D-003 [40, 41] have contributed, at least partially, to our results.

Osteoclasts have shown to promote the loss of osteochondral integrity and induce te extension of channels from bone marrow to the cartilage, and the subsequent exposure of subchondral nerves to proinflammatory and algesic factors from the synovial fluid.[42] Then, it makes sense to suppose that the antiresorptive effect of D-003 is relevant for ameliorating OA damage, since D-003 has been shown to prevent ovariectomy-induced bone loss and bone resorption in rats by the increase of osteoclast apoptosis.

It should be noted that complementary treatments have been useful to manage OA symptoms. Glucosamine sulfate has been suggested to have a carryover effect like disease-modifying agents, and the long-term treatment with this agent may lower the dependence of NSAID and delay OA progression,[43] and the use of chondroitin sulfate has been supported to manage symptomatic knee OA.[44]

D-003 treatment was safe and well tolerated, consistent with previous short and long-term clinical studies. [22, 23, 41]It should be noted that gastrointestinal AE were not referred by study subjects.

The present data suggest that D-003 treatment (10 – 20 mg/day) could be useful to manage OA symptoms, but this appreciation is preliminary. Indeed, taking into account that pain and impaired mobility are common OA symptoms that affect the life of the sufferers, which currently account millions of people worldwide, [1, 2] and the current concern about the AE induced by the non-selective NSAIDs and COX-2 inhibitors, the present results encourage further clinical research on the short and long-term effects of D-003 on subjects with OA, including long-term studies in different OA subtypes.

CONCLUSION

The results of this study indicate that D-003 (10 – 20 mg/day) for 6 weeks ameliorated osteoarthritis symptoms, and that treatment was well tolerated. Then, D003 could be beneficial for subjects suffering of OA, but this appreciation merits further clinical investigation.

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