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Effects of D-003, a Mixture of High-Molecular-Weight Sugar Cane Wax Acids, on Lipid Peroxidation Markers in Older Individuals: A Randomized, Double-Blind, Placebo-Controlled Study

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ABSTRACT

BACKGROUND: Aging is associated with increased lipid peroxidation (LP). D-003, a mixture of long-chain aliphatic primary acids purified from sugar cane wax, has been found to inhibit LP in experimental models and in healthy subjects.

OBJECTIVES: The aim of this study was to assess the effects of D-003 on LP markers and the lipid profile of older individuals.

METHODS: This randomized, double-blind, placebo-controlled study was conducted at the Plaza Veterans' House, Havana City, Cuba. Male and female patients aged ≥60 years with total cholesterol values of <6.1 mmol/L were eligible for inclusion in the study. After a 3-week lead-in and baseline assessment period, patients were randomized to receive PO D-003 5 mg/d, D-003 10 mg/d, or placebo for 8 weeks. The effect on copper-induced LP of low-density lipoprotein (LDL) particles was the primary variable, and the effects on plasma total antioxidant status (TAS), plasma malondialdehyde (MDA) concentration, plasma antioxidant enzyme (superoxide dismutase and glutathione peroxidase) activities, and the lipid profile were secondary variables. A clinical examination was performed at each visit (baseline, weeks 4 and 8). A clinical examination, LP, and blood tests (lipid profile, hematologic, and blood biochemistry safety indicators) were performed at baseline and after 8 weeks of treatment. Compliance and adverse events (AEs) were assessed at weeks 4 and 8. A 2-tailed $P < 0.05$ was considered statistically significant for comparisons of both continuous and categoric variables.

RESULTS: Fifty-four patients aged ≥60 years were assessed for inclusion in the study, and 51 patients (40 women, 11 men; mean [SD] age, 67 [6] years) were included in the study. The lag phase of conjugated diene formation increased significantly and in a dose-dependent manner in the group treated with D-003 5 mg (24.7%; $P < 0.01$) and in the group treated with D-003 10 mg (29.3%; $P < 0.01$) compared with placebo.

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The maximal rate of conjugated diene propagation decreased significantly in the D-003 5- and 10-mg groups (–22.7% and –25.8%, respectively; both, $P < 0.05$) compared with placebo. TAS increased significantly (17.7% and 23.0%, respectively; both, $P < 0.01$) in both active treatment groups compared with placebo. Plasma MDA concentration decreased significantly in the D-003 10-mg group (–28.6%; $P < 0.05$) but not in the D-003 5-mg group, compared with placebo. These changes were also significant compared with baseline. Antioxidant enzyme activities did not change in the active treatment groups compared with placebo or baseline. In the D-003 5- and 10-mg groups, significant decreases were found in LDL cholesterol concentration (–15.8% and –23.8%, respectively; both, $P < 0.001$) and total cholesterol concentration (–13.0% and –16.8%, both, $P < 0.05$) compared with placebo. High-density lipoprotein cholesterol concentration increased significantly in the D-003 5-mg group (5.7%; $P < 0.05$) and the D-003 10-mg group (18.2%; $P < 0.001$) compared with placebo. Changes in the lipid profile were also significant compared with baseline. In the placebo group, no variable changed significantly compared with baseline. D-003 was well tolerated at both dose levels, and no patient withdrew from the study. There were a total of 3 AEs reported: insomnia and acidity in 2 patients receiving placebo; and heartburn in 1 patient receiving D-003 5 mg.

CONCLUSIONS: D-003 5 and 10 mg/d administered to these older individuals (aged ≥60 years) for 8 weeks inhibited LP of LDL and increased TAS in a dose-dependent manner, while plasma MDA concentration decreased in the patients receiving D-003 10 mg/d only. D-003 was well tolerated at both doses. (*Curr Ther Res Clin Exp.* 2008;69:36–48) © 2008 Excerpta Medica Inc.

KEY WORDS: D-003, lipid peroxidation, antioxidant compounds, lipid-lowering, elderly, sugar cane wax acids.

INTRODUCTION

Because of increased life expectancy, elderly individuals are currently the fastest growing segment of the population worldwide.[1] Atherosclerotic diseases (eg, coronary heart disease [CHD]) and cerebrovascular diseases are the main causes of mortality in older individuals.[2] An estimated 7.6 million people died from CHD and 5.7 million from stroke in 2005.[2] Elevated serum low-density lipoprotein cholesterol (LDL-C) concentrations increase coronary risk[3,4]; but lowering LDL-C reduces coronary events in adults,[5–8] including the elderly.[8] Although the benefit of lowering cholesterol concentration in older individuals has been questioned,[9] the absolute risk of morbidity and mortality from CHD increases with age, and a high LDL-C concentration has been found to be a strong predictor of absolute coronary risk in the elderly[10]; the segment of the population with the highest rate of CHD. Updated guidelines[11] on cholesterol recommend management of serum LDL-C concentration in these individuals.

In addition to the pivotal role of increased LDL-C concentration as an atherogenic factor, lipid peroxidation (LP) of LDL results in the formation of oxidized LDL (LDL-o).

LDL-o is more atherogenic than LDL itself because it is scavenged by macrophages in an uncontrolled manner, triggering foam cell formation, which is the first step in

the formation of atherosclerotic lesions, and also because LDL-o is immunogenic and cytotoxic to the endothelium.[12] Considering the high frequency of atherosclerotic diseases in the elderly and the oxidative hypothesis of the aging process, treatments that concurrently prevent LDL oxidation and reduce serum LDL-C concentration might help prevent atherosclerosis and associated complications in this population.

D-003 is a mixture of long-chain aliphatic primary acids purified from sugar cane wax. Octacosanoic acid is the most abundant component of D-003; followed by triacontanoic, dotriacontanoic, and tetratriacontanoic acids, and lower concentration acids tetracosanoic, pentacosanoic, hexacosanoic, heptacosanoic, nonacosanoic, hentriacontanoic, tritiacontanoic, pentatriacontanoic, and hexatriacontanoic, all of which are within reproducible limits.[13]

D-003 has been found to inhibit LP in experimental models,[14–17] to increase the lag phase of conjugated diene formation, and to decrease the maximal rate (V_{max}) of conjugated diene propagation of LDL copper-induced peroxidation in healthy volunteers.[18] D-003 has also been associated with cholesterol-lowering effects in experimental and clinical studies.[19,20] Other studies[21,22] have found that D-003 inhibits cholesterol synthesis prior to mevalonate formation through the regulation of hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA) activity in fibroblasts cultured in a lipid-depleted medium.[21] D-003 was found to decrease plasma LDL-C concentration in rabbits with hypercholesterolemia associated with a casein-rich, fat-free diet.[21] D-003 was well tolerated in clinical trials,[19,20] and toxicologic studies[23–27] have not found treatment-related toxicity.

The primary aim of this study was to assess whether D-003, administered at doses of 5 and 10 mg/d for 8 weeks, favorably changed LP markers in older individuals. The secondary aim of this study was to assess the effects of D-003 on the lipid profile.

SUBJECTS AND METHODS

STUDY DESIGN

This randomized, double-blind, placebo-controlled study was conducted at the Plaza Veterans' House, Havana City, Cuba. The study protocol was approved by the Veterans' House independent ethics committee.

Volunteers were recruited by in-house referrals at the outpatient department of the Plaza Veterans' House Surgical Medical Research Center. Patients were enrolled into the study after provided informed written consent. General data regarding sex, age, height, weight, personal history, and family history were collected and recorded, and subjects underwent a complete physical examination, including pulse rate and arterial pressure and laboratory tests (hemoglobin, hematocrit, blood red cells, white blood cell and platelet counts, lipid profile and blood biochemistry safety indicators [fasting blood glucose, creatinine, and activities of aspartate and alanine aminotransferases], alkaline phosphatase, and creatine phosphokinase). Patients were instructed to adhere to a 3-week lead-in cholesterol-lowering diet based on therapeutic lifestyle change recommendations. After this lead-in period, patients were randomized to receive D-003 5 mg/d, 10 mg/d, or placebo for 8 weeks. A complete clinical examination was conducted at each visit. LP and blood tests (lipid profile, hematologic, and blood biochemistry safety indicators) were performed at baseline and after 8 weeks of treatment, while clinical

examination was performed, and compliance and adverse events (AEs) were assessed at weeks 4 and 8.

Patients

Male and female outpatients aged ≥60 years with total cholesterol values of <6.1 mmol/L were eligible for inclusion in the study. After the lead-in period, patients were assessed and had to meet the following serum lipid profile criteria: total cholesterol (TC) <6.1, LDL-C ≥3.4, and triglycerides (TG) <4.52 mmol/L. Patients with active renal or hepatic disease, diagnosed neoplastic diseases, uncontrolled hypertension (diastolic pressure ≥100 mm Hg), or diabetes mellitus (fasting serum glucose concentration ≥7.5 mmol/L), and those who had used antioxidants within 3 months prior to the study were also excluded. Patients who had experienced unstable angina, myocardial infarction, stroke, transient ischemic attacks, coronary surgery, or who were hospitalized due to other critical events (eg, revascularization surgery or any major surgery) in the 6 months prior to the study were excluded as well.

D-003 and placebo were identical in packaging. The treatments were coded by a computer-generated randomization scheme Clinstat (Martin Bland, London, United Kingdom), utilizing balanced blocks and a half-allocation ratio. Treatment tablets were administered once daily with the evening meal for 8 weeks.

Concomitant consumption of supplements with antioxidant effects (ie, vitamin E, vitamin C, multivitamins containing vitamin E or C, and any flavonoid-rich supplements) or lipid-lowering drugs (ie, statins, fibrates, niacin, ionic-exchange resins, fish oil omega-3 fatty acids, and phytosterols) was not allowed during the study.

Response Variables

As LP markers, we assessed the effects of D-003 on LP of LDL (as per the effect on the kinetics of conjugated diene generation and propagation), as well as plasma total antioxidant status (TAS), plasma malondialdehyde (MDA) concentration, and plasma antioxidant enzyme (superoxide dismutase [SOD] and glutathione peroxidase [GSHPX]) activities. The primary efficacy variable was the effect on LDL copper-induced LP. The treatment was considered to have beneficial effects on LP if there was a significant increase in the length of the lag phase of diene generation and/or a significant decrease in the V_{max} of ≥20% versus baseline and placebo. Effects on other LP markers and on the lipid profile were secondary efficacy variables. D-003 treatment was considered effective against these LP markers if plasma serum TAS increased and/or thiobarbituric acid-reactant substance (TBARS) generation decreased versus baseline and placebo. D-003 treatment was considered effective in lowering cholesterol if a decrease of ≥15% in LDL-C concentration versus baseline and placebo was determined.[28]

Assessment of Effects on Plasma Lipid Peroxidation Markers

After a 12-hour fast, venous blood samples were drawn again. Assays were conducted in triplicates.

Plasma lipid peroxide concentration was assayed by measuring the TBARS concentration.[29] A 0.5 mL of plasma was added to a solution of 0.2 mL of 8.1% sodium

dodecyl sulfate, 1.5 mL of 20% acetic acid adjusted to pH 3.5, and 1.5 mL of an aqueous solution of thiobarbituric acid (TBA). The mixture was diluted to 4 mL with distilled water and heated to 95°C. To prevent the formation of TBA reactants during the assay, 1 mM of butylated hydroxytoluene was added. After cooling, 1 mL of distilled water and 5 mL of n-butanol:pyridine (15:1 v/v) were added to the mixture, which was then mixed together and centrifuged at 4000 rpm for 20 minutes. The absorbance of the organic layer was measured at 532 nm. Freshly diluted MDA-bis (dimethyl acetal) was the reference standard. The concentration of lipid peroxide was reported as nmol of MDA/mL.

Plasma TAS in freshly drawn serum was determined using reagent kits (Randox Laboratories, Ltd., Crumlin, United Kingdom). This measurement is determined by the reaction between 2,2-azido-di-(3-ethylbenzthiazoline sulfonate) (ABTS), a peroxidase (metmyoglobin), and H_2O_2, which produces a blue-green radical cation (ABTS+) detected at 600 nm. The antioxidant potential of plasma samples added to the incubation mixture (0.01 mL) was determined according to color suppression and was expressed in mmol/L.

Whole plasma SOD activity was measured using reagent kits (Randox Laboratories, Ltd.) through the inhibition of the reaction of xanthine and xanthine oxidase with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to generate superoxide radicals, thereby producing formazan red.

GSHPX activity in heparinized whole blood samples was measured using reagent kits (Randox Laboratories, Ltd.). Since GSHPX catalyzes the oxidation of glutathione by cumene hydroperoxide, the enzyme activity was measured indirectly. Oxidized glutathione reacts with glutathione reductase, and the amount of reduced glutathione was estimated through the concomitant oxidation of nicotinamide adenine dinucleotide phosphate hydrogen to nicotinamide adenine dinucleotide phosphate at 340 nm. Enzyme activities were expressed as UI/mg Hb (SOD) and UI (GSHPX).

The effects of D-003 on the LP of LDL were assessed according to Kleinveld et al.[30] Samples were collected into plastic tubes with 10% ethylenediaminetetraacetic acid (EDTA) (final concentration 0.1%), and LDL was isolated by discontinuing density-gradient ultracentrifugation (22 hours at 285,000*g* [maximum]) in an ultracentrifuge SW40 rotor (Beckman Coulter, Fullerton, California). The kinetic of Cu^{2+}-induced LDL oxidation was measured according to Esterbauer et al.[31] Isolated LDL was exhaustively dialyzed against a 200-fold volume of EDTA-free phosphate-buffered saline (0.01 M phosphate, 0.15 M sodium chloride, and pH 7.4). After overnight dialysis, LDL was oxidized with copper sulfate$_4$ 5 μM, a final concentration, in a cell-free system, and the extent of LDL oxidation was determined by the amount of conjugated diene formation, which was measured by the absorbance changes at 234 nm at 37°C over a 5-hour period.

Data from conjugated diene generation were expressed as time course curves. The t lag (minutes) and V_{max} values (optical density [OD × 10^{-2}/min · mg of LDL protein) were estimated from the oxidation curve. The inter- and intra-assay CVs in our conditions have been previously described.[32] The LDL protein concentration was estimated using a modification of the Lowry method.[33]

Other Laboratory Tests

Serum TC and TG concentrations were measured using enzymatic reagent kits (Randox Laboratories, Ltd.), high-density lipoprotein cholesterol (HDL-C) concentration was measured as the amount of cholesterol in the supernatant after precipitating β-lipoprotein,[34] and LDL-C concentration was calculated using the Friedewald equation.[35]

Hematologic safety indicators were determined using the hematological complex equipment, while blood biochemistry indicators were determined through enzymatic methods, using reagent kits (Randox Laboratories, Ltd.) in a Hitachi 419 (Hitachi, Ltd., Tokyo, Japan). Both equipments were located at the clinical laboratory of the Surgical Medical Research Center.

Tolerability Assessments

Data from physical examination, laboratory analyses, and AEs were included in the tolerability analysis. Laboratory tolerability indicators included concentrations of hemoglobin, hematocrit, red blood cells, white blood cell and platelet counts, serum creatinine, fasting blood glucose, and serum activities of aspartate and alanine aminotransferases, alkaline phosphatase, and creatinine phosphokinase.

An *AE* was defined as any undesirable experience, whether or not it was considered treatment related. AEs were classified as: mild—discontinuation of treatment was not required; moderate—treatment had to be discontinued or specific treatment was required; and serious—event resulted in hospitalization or death.

Statistical Analysis

All data were analyzed using intent-to-treat analysis. To ensure a power of 80% based on the assumption that the highest dose should produce an increase in the lag phase and/or a reduction in V_{max} of ≥20% compared with placebo, with a 5% significance level, ≥15 patients were needed for each group. Additionally, to control for an ~10% dropout rate, 50 total patients were needed for the study.

The Wilcoxon test for paired samples was used to make within-group comparisons of continuous variables. The Mann-Whitney *U* test was used to make between-group comparisons. The Fisher exact test was used to compare categoric variables. A 2-tailed $P < 0.05$ was considered statistically significant. Statistical analyses were performed using Statistics for Windows version 4.2 (Statsoft Inc., Tulsa, Oklahoma).

RESULTS

Baseline Characteristics

Of the 54 patients eligible for inclusion in the study, 51 (40 women, 11 men; mean [SD] age, 67 [6] years) were included and randomized to one of the 3 groups. Three of the patients were excluded because of adverse laboratory findings (serum LDL-C [<3.4 mmol/L], 2; and fasting glucose [>7.5 mmol/L], 1). Treatment groups were matched with respect to demographics including concomitant treatment (**Table I**).

Effects on Lipid Peroxidation Markers

The LP markers were well balanced in all groups at randomization. After treatment, the LP markers were unchanged in the placebo group compared with baseline. The lag

phase of the conjugated diene formation increased significantly and in a dose-dependent manner in the group that received D-003 5 mg and the group that received D-003 10-mg compared with placebo (24.7% and 29.3%, respectively; $P < 0.05$ and $P < 0.01$, respectively) and baseline ($P < 0.01$ and $P < 0.05$, respectively). The V_{max} of conjugated diene propagation decreased significantly in the D-003 5- and 10-mg groups compared with placebo (–22.7% and –25.8%, respectively; both, $P < 0.01$) and with baseline (both, $P < 0.05$) (Table II).

TAS increased significantly and in a dose-dependent manner in the D-003 5- and 10-mg groups compared with placebo (17.7% and 23.0%, respectively; both, $P < 0.05$) and with baseline (both, $P < 0.01$). Plasma MDA concentration decreased significantly in the D-003 10-mg group compared with placebo (–28.6%; $P < 0.05$) and with baseline ($P < 0.05$). D-003 5 mg was not associated with significant changes in MDA concentration compared with placebo or baseline. Plasma SOD and GSHPX activities did not change in either treatment group (Table II).

EFFECTS ON LIPID PROFILE

At baseline, lipid profile variables were not statistically different between groups. No significant changes from baseline were found in the placebo group. After 8 weeks

Table I. Baseline demographic and clinical characteristics of patients (N = 51).*

Characteristic	D-003 5 mg/d (n = 17)	D-003 10 mg/d (n = 17)	Placebo (n = 17)
Age, mean (SD), y	69 (6)	66 (5)	67 (7)
Sex, no. (%)			
Female	14 (82.4)	13 (76.5)	13 (76.5)
Male	3 (17.6)	4 (23.5)	4 (23.5)
Body mass index, mean (SD), kg/m^2	26.2 (2.2)	25.7 (2.8)	25.3 (2.6)
Personal history, no. (%)			
Hypertension	11 (64.7)	10 (58.8)	10 (58.8)
Smoking	2 (11.8)	2 (11.8)	2 (11.8)
Diabetes mellitus	1 (5.9)	1 (5.9)	2 (11.8)
Concomitant therapy,† no. (%)			
Diuretics	6 (35.3)	6 (35.3)	5 (29.4)
Angiotensin-converting enzyme inhibitors	2 (11.8)	3 (17.6)	2 (11.8)
β-Blockers	2 (11.8)	2 (11.8)	2 (11.8)
Calcium channel blockers	1 (5.9)	1 (5.9)	2 (11.8)
Oral hypoglycemic drugs	1 (5.9)	1 (5.9)	1 (5.9)
Anxiolytics	0	2 (11.8)	1 (5.9)

*There were no significant between-group differences.
†Drugs used by ≥3 subjects.

Table II. V_{max} of diene formation on the effects of D-003 5 and 10 mg/d on lipid peroxidation (LP) markers in older individuals by treatment group. Data are mean (SD).

	Baseline	8 Weeks	% Change
Copper-induced LP of LDL T lag, min			
Placebo	85.99 (16.49)	81.37 (24.28)	–5.4
D-003 5 mg/d	85.70 (21.33)	106.90 (30.66)*†	24.7†
D-003 10 mg/d	88.20 (23.53)	114.05 (30.82)†‡	29.3§
Propagation rate, OD × 10^{-2}/min · mg of LDL protein			
Placebo	21.66 (8.42)	21.65 (6.91)	–0.0
D-003 5 mg/d	19.40 (7.00)	15.00 (5.66)*§	–22.7§
D-003 10 mg/d	21.95 (11.36)	16.29 (5.76)*†	–25.8§
Total antioxidant status, mmol/L			
Placebo	0.82 (0.13)	0.80 (0.19)	–2.4
D-003 5 mg/d	0.79 (0.15)	0.93 (0.20)‡	17.7†
D-003 10 mg/d	0.78 (0.16)	0.96 (0.21)†‡	23.0†
Plasma malondialdehyde, nmol/mL			
Placebo	62.47 (17.75)	57.74 (24.27)	–7.6
D-003 5 mg/d	65.33 (17.28)	58.51 (20.73)	–10.4
D-003 10 mg/d	69.53 (17.52)	49.62 (14.23)*	–28.6†
Plasma superoxide dismutase, UI/mg hemoglobin			
Placebo	120.91 (41.48)	105.87 (44.66)	–
D-003 5 mg/d	133.55 (35.16)	121.63 (57.76)	–
D-003 10 mg/d	128.84 (24.18)	133.93 (50.04)	–
Plasma glutathione peroxidase, UI			
Placebo	143.84 (41.08)	177.32 (56.90)	–
D-003 5 mg/d	160.61 (41.72)	159.60 (39.55)	–
D-003 10 mg/d	159.88 (55.50)	167.14 (35.32)	–

LDL = low-density lipoprotein; OD = optical density.
*$P < 0.05$ versus baseline (Wilcoxon test for paired samples).
†$P < 0.05$ versus placebo (Mann-Whitney U test).
‡$P < 0.01$ versus baseline (Wilcoxon test for paired samples).
§$P < 0.01$ versus placebo (Mann-Whitney U test).

of treatment with D-003 5 and 10 mg, LDL-C concentration decreased significantly compared with placebo (–15.8% and –23.8%, respectively; both, $P < 0.001$) and with baseline (both, $P < 0.001$). TC concentration also decreased significantly compared

with placebo (–13.0% and –16.8%; both, $P < 0.05$) and with baseline (both, $P < 0.001$). HDL-C concentration increased significantly in the D-003 5-mg group and the D-003 10-mg group compared with placebo (5.7% and 18.2%, respectively; $P < 0.05$ and $P < 0.001$, respectively) and baseline ($P < 0.05$ and $P < 0.001$, respectively). TG decreased modestly but significantly in the D-003 10-mg group compared with placebo and baseline ($P < 0.05$) (Table III).

TOLERABILITY

Treatment was well tolerated at both dose levels. D-003 did not adversely affect physical or blood chemistry findings (data not shown). There were no abnormal laboratory results, and no patient withdrew from the study. A total of 3 patients experienced mild AEs—insomnia and acidity (1 patient for each in the placebo group) and heartburn (1 patient in the D-003 5-mg group).

DISCUSSION

This study suggests that treatment with D-003 5 and 10 mg for 8 weeks was associated with dose-dependent beneficial effects on LP markers in older individuals that were consistent with previous data.[18,21]

Table III. Effects of D-003 5 and 10 mg/d on the serum lipid profile in older individuals by treatment group. Data are mean (SD).

Treatment	Baseline	8 Weeks	% Change
LDL-C, mmol/L			
Placebo	4.02 (0.22)	4.03 (0.19)	0.2
D-003 5 mg/d	4.04 (0.33)	3.40 (0.15)*†	–15.8†
D-003 10 mg/d	3.99 (0.26)	3.04 (0.14)*†	–23.8†
Total cholesterol, mmol/L			
Placebo	5.73 (0.29)	5.76 (0.26)	0.5
D-003 5 mg/d	5.75 (0.28)	5.00 (0.11)*†	–13.0‡
D-003 10 mg/d	5.71 (0.26)	4.75 (0.12)*†	–16.8‡
HDL-C, mmol/L			
Placebo	1.08 (0.18)	1.04 (0.13)	–3.7
D-003 5 mg/d	1.05 (0.18)	1.11 (0.13)§	5.7‡
D-003 10 mg/d	1.10 (0.21)	1.30 (0.16)*†	18.2†
Triglycerides, mmol/L			
Placebo	1.71 (0.63)	1.80 (0.49)	5.3
D-003 5 mg/d	1.81 (0.72)	1.69 (0.38)	–6.6
D-003 10 mg/d	1.83 (0.55)	1.63 (0.32)§	–10.9‡

LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.
*$P < 0.001$ versus baseline (Wilcoxon test for paired samples).
†$P < 0.001$ versus placebo (Mann-Whitney U test).
‡$P < 0.05$ versus placebo (Mann-Whitney U test).
§$P < 0.05$ versus baseline (Wilcoxon test for paired samples).

The groups were well matched at baseline in terms of demographic and clinical characteristics. Although 40 of the patients (78.4%) were women, it reflected the composition of the population attending the clinic. The percentage of women was similar in the 3 groups. Because the study groups were homogeneous at baseline and the LP markers were unchanged in the placebo group, the effects discussed here were probably associated with the treatment.

At both doses, oral D-003 protected LDL particles against LP, as supported by the increase in the lag phase and the decrease in the V_{max} of the conjugated diene generation time course of LDL copper-dependent LP. In vitro generation of conjugated dienes with copper was an adequate surrogate of oxidative stress in humans and correlated well with estimated in vivo LDL oxidation.[36] In particular, evidence supports a direct relationship between the susceptibility of LDL to in vitro oxidation and atherogenic risk.[37]

The aging process might enhance oxidative stress, leading to increased susceptibility of LDL to peroxidation,[38] with LDL-o triggering atherosclerosis and complications.[12] Therefore, the preventive effect of D-003 on the LP of LDL particles at doses that are also effective for lowering LDL-C is potentially beneficial to older individuals.

Beyond the effects of D-003 on LP of the LDL molecule, the primary efficacy variable, D-003 also increased plasma TAS, which reflects the total antioxidant potential of plasma against oxidative attack by free radicals. This suggests that the water-soluble and nonwater-soluble antioxidant capacity of plasma might be increased with D-003.

D-003 at 10 mg/d, but not at 5 mg/d, decreased the plasma concentration of MDA (a final product of LP breakdown), as measured by TBARS concentration. Nevertheless, because other compounds might interfere with the results, the specificity of measuring plasma MDA concentration using TBARS concentration is not sufficient.[39] Further studies are needed to investigate the effects of D-003 on plasma MDA concentration using direct estimation of MDA.

The moderate effects of D-003 5 mg and 10 mg on serum LDL-C and TC concentrations found in this study, which were consistent with those reported previously,[17–19] have been associated with the inhibition of cholesterol synthesis before mevalonate formation through the regulation of HMG-CoA reductase activity and the increase in plasma LDL clearance.[21,22] However, the mechanism by which D-003 increases serum HDL-C is unknown.

D-003 10 mg/d (but not 5 mg/d) significantly reduced serum TG concentration compared with baseline ($P < 0.05$), while no such reduction was found in TG concentration in other studies of D-003.[15–20] Nevertheless, since the change was not significant versus placebo, it should not be considered meaningful. However, we believe that further studies should explore whether this effect occurs with higher doses and after longer treatment periods.

We found that D-003 protected against copper-induced LP of LDL in vitro in a cell-free system, which included only factors intrinsic to LDL. However, the susceptibility of LDL to in vivo oxidation is also influenced by extrinsic factors in the LDL microenvironment, including the presence of native HDL.[40] Although the protective effects of HDL against coronary risk were first thought to be associated with the removal of excess cholesterol from peripheral tissues via reverse cholesterol transport,[41] recent

data[42] suggest that HDL protects against atherosclerosis through other mechanisms, since LDL oxidation is prevented by the action of 2 HDL-associated enzymes, paraoxonase and arylesterase, which reduce the deleterious effects of LP on LDL. Considering that D-003 has been found to produce moderate to marked increases in serum HDL-C concentration in both experimental and clinical studies,[14–19] we cannot disregard the possibility that its antioxidant effects are linked to such action. However, because identifying the antioxidant mechanisms of D-003 was beyond the objectives of this study, this is merely speculative.

CONCLUSIONS

D-003 5 and 10 mg/d administered for 8 weeks inhibited the LP of LDL and increased TAS in a dose-dependent manner, while D-003 10 mg/d also reduced plasma MDA without modifying antioxidant enzyme activities. The treatment also lowered LDL-C and TC, raised HDL-C, and was well tolerated.

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