Effect of D-003, a mixture of very high molecular weight aliphatic acids purified from sugarcane wax, on serum lipid peroxidation (LP) markers in elderly.

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ABSTRACT: Background: D-003 is a mixture of long-chain aliphatic primary acids purified from sugar cane wax with cholesterol-lowering and preventive effects on lipoprotein lipid peroxidation (LP) proven in experimental and clinical studies.

Objectives: To investigate if D-003 favourably modifies LP markers on older individuals.

Methods: This double-blinded, randomized, placebo-controlled study investigated the effects of D-003 (5 and 10 mg/d) administered for 8 weeks on serum LP markers of older individuals. We assessed the effects of D-003 on LP of LDL as primary variable, while effects on plasma total antioxidant status (TAS), malondialdehyde (MDA) levels and plasma antioxidant enzyme activities were also investigated. We investigated the effects on lipid profile. Fifty-one older individuals (40 women, 11 men) were randomized to placebo or D-003 (5 or 10 mg/d), tablets being taken once a day with evening meal for 8 weeks. Laboratory tests and physical examination were performed at baseline and after 4 and 8 weeks on therapy, whereas compliance and adverse experiences (AE) were assessed at weeks 4 and 8.

Results: D-003 (5 and 10 mg/d) significantly increased (p < 0.05 and p < 0.01) lag phase by 24.7 % and 29.3 %, respectively, while decreased propagation rate (p< 0.05) by 22.7 % (5 mg/d) and 25.8 % (10 mg/d). D-003 (5 and 10 mg/d) increased (p < 0.01) TAS by 17.7% and 23.0%, respectively, but only the changes achieved with 10 mg/d were different from placebo (p < 0.05). D-003 at 10 mg/d, not at 5 mg/d, significantly lowered (p < 0.05) plasma levels of thiobarbituric acid reactive substances (TBARS). D-003 (5 or 10 mg/d) did not modify superoxide dismutase (SOD) and GPX (glutathione peroxidase) activities. Also, D-003 (5 and 10 mg/d) reduced significantly low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and increased high-density lipoprotein-cholesterol (HDL-C). D-003 (10 mg/d) also reduced modestly, but significantly triglycerides. No significant changes of LP or lipid profile occurred in placebo. Treatment was well tolerated. D-003 did not impair safety indicators. No subject withdrew from the study. Only 3 subjects (2 placebo, 1 D-003 5 mg/d) reported AE: the two placebo patients reported insomnia and acidity, respectively, while that treated with D-003 referred acidity.

Conclusions: D-003 (5 and 10 mg/d) administered for 8 weeks to older subjects effectively prevented LP in older and favourable modified lipid profile parameters. The present results add new data on the antioxidant effects of D-003, including demonstration on a new population subset. However, this aspect deserves further investigation including studies with larger sample sizes, doses and treatment duration.

Key words: D-003, older subjects, elderly, cholesterol-lowering drugs, antioxidant compounds, lipid peroxidation.

INTRODUCTION

Human aging is a complex biological process involving multiple mechanisms. The elderly is the fastest growing population as results from the increase on life expectancy, and coronary heart disease (CHD), cerebrovascular and peripheral artery disease (PAD) are the major causes of mortality and morbidity in older individuals.

Epidemiological studies showed a direct relationship between elevated low-density lipoprotein cholesterol (LDL-C) and CHD, and the benefits of lowering LDL-C for preventing coronary events in adults. Hypercholesterolemia (HC) treatment in the elderly, however, was questioned because of the contribution of elevated serum LDL-C as predictor of the relative coronary risk decreases with age.
Nevertheless, high LDL-C levels still are strong predictors for absolute coronary risk in the elderly, a population with the highest rate of CHD. Also, strata analysis performed in early statin trials showed the clinical benefits of lowering cholesterol in the elderly and 5,806 of 20,536 patients included in the Heart Protection Study were aged ≥70 years, the benefits of lowering LDL-C on clinical outcomes of such population being demonstrated. Finally, PROSPER, the first study examining the cardiovascular benefits of a statin in older subjects corroborated such benefits. Then, recent guidelines for HC management have included older individuals as specific target.

Although increased LDL-C levels are a major coronary risk factor, other triggering processes are associated to atherosclerosis, the pathological process involved in the etiology of occlusive vascular diseases. Among them, lipid oxidation (LP) of LDL plays a key role in atherosclerosis development. Oxidized LDL (LDLo) is highly atherogenic, being uncontrolled scavenged by macrophages, forming foam cells. Also, LDLo is chemotactic to circulating monocytes and T cells, immunogenic and cytotoxic to endothelial wall.

Thus, not only elevated levels, but also the oxidation status of LDL is relevant for the development of atherosclerosis and its consequences. Considering the high frequency of atherosclerotic diseases in the elderly, such association supports the importance of the oxidative hypothesis of aging process. Hence, drugs lowering LDL-C levels and preventing LDL oxidation could be promising to prevent atherosclerosis complications in the elderly.

D-003 is a mixture of higher aliphatic primary acids purified from sugar cane (Saccharum officinarum, L) wax, octacosanoic acid being its main component, followed by triacontanoic, dotriacontanoic, and tetratriacontanoic acids. Other long-chain fatty acids are present in lower proportion. Cholesterol lowering effects of D-003, characterized by the reduction of serum LDL-C and total cholesterol (TC), with concomitant increase of high-density lipoprotein-cholesterol (HDL-C) have been proven in experimental models, healthy volunteers and patients with Type II hypercholesterolemia. Experimental evidence suggests that D-003 lowers cholesterol trough and inhibition of cholesterol biosynthesis associated to a down regulation of HMG-CoA reductase enzyme activity. Also, D-003 increased plasma LDL clearance in rabbits with endogenous hypercholesterolemia induced with a casein-rich, fat-free diet.

Experimental and clinical studies have shown that oral treatment with D-003 also inhibits LP. Thus, D-003 inhibited tissue and lipoprotein metal and non-metal dependent LP in rats. D-003 significantly increased lag time (t lag) and decreased conjugated diene generation maximal rate (Vmax), the production of thiobarbituric acid reactive substances (TBARS) induced with A 2′2′-azo-bis-2-amidino-propane (AAPH) and the generation of carbonyl groups of rat plasma lipoprotein (VLDL + LDL) and attenuated the reduction of lysine reactivity.

A previous double-blinded, placebo-controlled clinical trial showed that D-003 administered at 5 or 10 mg/day for 8 weeks, significantly inhibited “in vitro” LP of LDL particles, while showed cholesterol-lowering effects on human healthy volunteers. Toxicological studies did not shown D-003-related oral toxicity, even at 1 g/kg administered for > 6 months. Then, the potential risk to be used at doses < 1 mg/kg in clinical studies is low.

MATERIALS AND METHODS

Study design
This randomized, double blinded, parallel group study was conducted at the Medical Surgical Research Center. The patients were enrolled at Plaza Veterans’ House (Havana City, Cuba). The independent Ethic Committee from the Medical Surgical Research approved the study protocol. Patients were enrolled after given the informed written consent. At recruitment (visit 1), general data were recorded and subjects underwent a clinical examination. Eligible individuals were randomized to placebo or D-003 5 or 10 mg/day. Participants were advised to take study drugs once a day with the evening meal for 8 weeks.

LPO, blood tests, clinical examination and request for adverse experiences (AE) were performed at baseline and after 8 weeks of therapy.
Study participants
Outpatients of both sexes aged ≥ 60 years were enrolled in the study. To be included in the study, subjects should show serum TC < 6.0 mmol/L at recruitment, so that only subjects with normal or mildly elevated TC levels should be included.
Subjects with active renal or hepatic diseases, diagnosed neoplastic disease, uncontrolled hypertension (diastolic pressure ≥ 100 mmHg) or diabetes mellitus (serum glucose ≥ 7.5 mmol/L) were excluded from the study. Exclusion criteria also included patients with history of unstable angina, myocardial infarction, stroke, transient ischemic attacks, coronary surgery or hospitalizations due to critical events occurred in the 6 months prior to study and subjects consuming antioxidant agents within the last 3 months before study recruitment.
Concomitant medications
Consumption of any drug or supplement with recognized lipid modifying and/or antioxidant effect was not allowed during the study.
Response variables
Effects on LP of LDL were considered as the primary efficacy variables. Then, D-003 was considered effective if significantly increased lag and decreased Vmax of conjugated diene kinetics of LDL copper-induced LP in more than 20 % compared with baseline and placebo. In addition, effects on other LP markers were considered as secondary response variables. For that, D-003 was considered as effective if increased serum TAS and reduced MDA concentration and TBARS generation compared with baseline and placebo.
In this study, effects of D-003 on lipid profile were evaluated as secondary and tertiary response variables. Thus, decreases on LDL-C were secondary efficacy outcomes, the treatment being considered effective only if serum LDL-C was significantly reduced by at least 15% respect to baseline. 42 Other lipid profile variables were tertiary efficacy variables.
Tolerability
Data from physical examination, laboratory analysis and AE interviews were included for the analysis of drug tolerability. Laboratory safety indicators included determination of hemoglobin, creatinine, glucose, aspartate (ASAT) and alanine aminotransferases (ALAT).
An AE was any undesirable experience occurred to a trial subject during the study, independently if they were or not drug-related. AE were predefined as “mild” if did not require therapy stopping, “moderate” when required therapy discontinuation according to physician criterion and/or specific treatment of AE, and “serious” when leads to hospitalization or death.
Assessment of effects on plasma LP markers
Lipid peroxides levels in plasma were assayed by measuring TBARS levels. 43 Briefly, 0.5 ml of plasma were added to a solution of 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid previously adjusted to pH 3.5 and 1.5 ml of aqueous solution of thiobarbituric acid (TBA). The mixture was diluted up to 4 ml with distilled water and heated at 95 °C. To prevent the production of TBA reactants during the assay, 1mM of BHT was added to each mixture. After cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol:pipidine (15:1 v/v) were added and shaken vigorously. After centrifugation, the organic layer was taken and its absorbance at 532 nm was measured. Freshly diluted MDA-bis (dimethyl acetal) was used as reference standard. The levels of lipid peroxides were reported as nmol of MDA/ml, values being the means of three replies.
Plasma TAS was determined in freshly drawn serum using a kit (Randox Laboratories, Ltd., Crumlin, UK) in which the incubation of 2,2’-Azido-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (metamyoglobin) and H2O2 produces the radical cation ABTS*+. This specie is blue-green, being detected at 600 nm. The antioxidant potential of plasma samples added at the incubation mixture (0.01ml) was determined by colour suppression and expressed in mmol/L.
SOD activity was measured by a colorimetric kit from Randox Laboratories Ltd (Crumlin, UK), employing xanthine and xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form formazan red. The SOD activity of whole blood samples was determined by the degree of inhibition of this reaction and expressed in SOD units/g hemoglobin.
GSHPX activity was determined in whole blood heparinized samples with a reagent kit, also from Randox Laboratories. Since GSHPX catalyses the oxidation of glutathione by cumene hydroperoxide, the enzyme activity was indirectly measured, determining the conversion of oxidized glutathione formed in presence of glutathione reductase. The amount of reduced glutathione was estimated by concomitant oxidation of NADPH to NADP+ at 340 nm. Enzyme activity was reported in U/L.
Technique to assess LDL oxidation.
Venous blood samples were collected into plastic tubes containing the appropriate volume of 10% EDTA to obtain a final concentration of 0.1%, LDL was isolated by discontinue density-gradient
ultracentrifugation (22 h at 285 000 g(max) in a Beckman SW40 rotor in a Beckman ultracentrifuge according to Kleinveld et al., 44

**Kinetic of Cu²⁺ induced oxidation of LDL.** Experiments were performed according to Esterbauer et al. 45 The isolated LDL was exhaustively dialyzed against 200-fold volume of phosphate-buffered saline (PBS, 0.01 M phosphate, 0.15 M NaCl, pH 7.4) EDTA-free. Following overnight dialysis, LDL was oxidized in a cell-free system in the presence of CuSO₄ 5 µM (final concentration). The degree of LDL oxidation was measured with respect to formation of conjugated dienes, by monitoring the change in absorbance at 234 nm (26), in triplicate experiments performed over 5 h at 37°C. Data derived from the conjugated diene generation were expressed as time courses curves. From the oxidation curve, the lag time (min) and the maximal rate of oxidation (D.O> x 10⁻² per minutes per mg of LDL protein). The inter- and intra-assay coefficients of variation in our experimental conditions have been previously described. 46 LDL concentration was estimated by determining protein concentration by a modification of the Lowry procedure. 47

**Other laboratory tests**

Blood venous samples were taken after 12-h overnight fast for laboratory tests. Serum levels of TC and triglycerides were determined by enzymatic methods using reagents kits from Randox (Randox, Crumlin, UK). Levels of HDL-C were determined according to cholesterol content of the supernatant obtained after precipitation of β-lipoprotein. 48 LDL-C was calculated using the Friedewald equation. 49

**Statistical analysis**

All data were analysed according to the intention to treat approach. Sample size was calculated on the assumption that differences between increases on t lag and/or reduction of Vmax of conjugated diene kinetics in the group treated with the highest doses of D-003 would be >20% with respect to the change occurring in placebo. For determining such difference with 80% of test power and 5% of significance level, a sample size of 15 patients/arm would be sufficient. Within-groups comparisons of continuous variables were performed using the Wilcoxon test for paired samples; between groups comparisons were made using the Mann Whitney U-test. Comparisons of categorical variables were done using the Fisher’s Exact Test. A two-tailed probability value < 0.05 was considered as statistically significant. Statistical analyses were performed using Statistic for Window package program.

**RESULTS**

**Patients characteristics**

On the 54 recruited, 51 eligible subjects were included in the study. Table 1 shows that study groups were well matched with respect to baseline characteristics. Most included subjects were women (40/51, 78.4%). No included subject was obese (kg/m² ≥ 30). Most study subjects (51) showed mild HC since TC values were from 5.0 to 5.6 mmol/L, while the rest showed values below 5.0 mmol/l, this condition being also well matched in all groups.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of study patients</th>
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<tr>
<td></td>
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<tr>
<td>Age (years) (X ± SD)</td>
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<tr>
<td>Body mass index (kg/m²) (X ± SD)</td>
</tr>
<tr>
<td>Gender:</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
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<tr>
<td>Personal history of:</td>
</tr>
<tr>
<td>Hypertension</td>
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<tr>
<td>Smoking</td>
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<td>Diabetes mellitus</td>
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<td>Concomitant therapy</td>
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<tr>
<td>Diuretics</td>
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<tr>
<td>ACEI</td>
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<tr>
<td>β-blockers</td>
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<tr>
<td>Calcium channel blockers</td>
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<tr>
<td>Oral hypoglycemic drugs</td>
</tr>
<tr>
<td>Anxiolytics</td>
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</tbody>
</table>

n number of patients, X mean, SD standard deviation, ACEI angiotensin converting enzyme inhibitors

*The table only includes concomitant drugs consumed by at least 3 study patients

All comparisons were not significant (Mann Whitney U test for continuous variables and Fisher Exact Probability test for categorical variables)
Effects on LP markers
Table 2 shows the effects of D-003 on LP parameters. At baseline all groups showed similar values of LP markers. D-003 at 5 and 10 mg/day significantly changed the primary efficacy variable respect to baseline and placebo. Thus, D-003 at 5 and 10 mg/day significantly increased lag phase (p < 0.05) by 24.7 % and (p < 0.01) 29.3 %, respectively, while decreased Vmax of propagation rate (p< 0.05) by 22.7 % (5 mg/day) and 25.8 % (10 mg/day).

Table 2. Effects of D-003 on lipid peroxidation indicators of study patients (x ± SD)

<table>
<thead>
<tr>
<th>LDL oxidation</th>
<th>Baseline</th>
<th>8 weeks</th>
<th>% changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tlag (min)</td>
<td>Placebo</td>
<td>85.99 ± 16.49</td>
<td>81.37 ± 24.28</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>85.70 ± 21.33</td>
<td>106.90 ± 30.66</td>
<td>+ 24.7 +</td>
</tr>
<tr>
<td>10 mg/day</td>
<td>88.20 ± 23.53</td>
<td>114.05 ± 30.82</td>
<td>+ 29.3 ++</td>
</tr>
<tr>
<td>Propagation rate (O.D x 10⁻²/min)</td>
<td>Placebo</td>
<td>21.66 ± 8.42</td>
<td>21.65 ± 6.91</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>19.40 ± 7.00</td>
<td>15.00 ± 5.66</td>
<td>-22.7 ++</td>
</tr>
<tr>
<td>10 mg/day</td>
<td>21.95 ± 11.36</td>
<td>16.29 ± 5.76</td>
<td>-25.8 ++</td>
</tr>
<tr>
<td>Total Antioxidant Status (TAS) (mmol/L)</td>
<td>Placebo</td>
<td>0.82 ± 0.13</td>
<td>0.80 ± 0.19</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>0.79 ± 0.15</td>
<td>0.93 ± 0.20</td>
<td>+ 17.7</td>
</tr>
<tr>
<td>10 mg/day</td>
<td>0.78 ± 0.16</td>
<td>0.96 ± 0.21</td>
<td>+ 23.0</td>
</tr>
<tr>
<td>Malondialdehyde (MDA) (TBARS, µMol)</td>
<td>Placebo</td>
<td>62.47 ± 17.75</td>
<td>57.74 ± 24.27</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>65.33 ± 17.28</td>
<td>58.51 ± 20.73</td>
<td></td>
</tr>
<tr>
<td>10 mg/day</td>
<td>69.53 ± 17.52</td>
<td>49.62 ± 14.23*</td>
<td></td>
</tr>
<tr>
<td>Superoxide dismutase (SOD) (UI/mg Hb)</td>
<td>Placebo</td>
<td>120.91 ± 41.48</td>
<td>105.87 ± 44.66</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>133.55 ± 35.16</td>
<td>121.63 ± 57.76</td>
<td></td>
</tr>
<tr>
<td>10 mg/day</td>
<td>128.84 ± 24.18</td>
<td>133.93 ± 50.04</td>
<td></td>
</tr>
<tr>
<td>Glutatione peroxidase (GPX) (UI)</td>
<td>Placebo</td>
<td>143.84 ± 41.08</td>
<td>177.32 ± 56.90</td>
</tr>
<tr>
<td>5 mg/day</td>
<td>160.61 ± 41.72</td>
<td>159.60 ± 39.55</td>
<td></td>
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<tr>
<td>10 mg/day</td>
<td>159.88 ± 55.50</td>
<td>167.14 ± 35.32</td>
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</table>

p < 0.05, ** p < 0.01, Comparison with baseline (Wilcoxon test for paired samples); + p < 0.05, ++ p < 0.01, Comparison with placebo (Mann Whitney U test)

Also, D-003 (5 and 10 mg/day) significantly increased (p < 0.01) TAS by 17.7% and 23.0%, respectively, compared with baseline, but only the changes achieved with 10 mg/day were different from placebo (p < 0.05). D-003 at 10 mg/day, not at 5 mg/day, significantly lowered (p < 0.05) TBARS plasma levels compared with baseline. D-003 at 5 or 10 mg/day, however, did not modify SOD and GPX activity. All LP markers remained unchanged in placebo group throughout the study.

Effects on lipid profile
Table 3 summarizes the effects of D-003 on lipid profile. At baseline, lipid profile variables were comparable in all groups. No significant changes of lipid profile variables occurred in placebo group. After 8 weeks on therapy, D-003 at 5 and 10 mg/day reduced significantly (p < 0.001 vs baseline and p < 0.0001 vs placebo) LDL-C (13.94% and 21.8%, respectively) and TC (10.0 % and 12.5 %). Also, D-003 increased HDL-C (p < 0.05, 5.7 %) (5 mg/day) and (p < 0.01, 18.5 %) compared with baseline, but only the differences obtained with 10 mg/day were different from placebo. D-003 at 10 mg/day reduced modestly, but significantly, triglycerides (10.9 %).

Tolerability
Treatment was well tolerated. D-003 did not impair any physical or blood safety indicators (data not shown). No subjects withdrew prematurely from the study. Only 3 subjects (2 placebo and 1 treated with D-003 5 mg/day) reported three mild AE. Thus, the two placebo patients reported insomnia and acidity, respectively, while the patient treated with D-003 5 mg/day reported acidity.
Table 3. Effects of D-003 (5 and 10 mg/day) on lipid profile (mmol/L) of study patients (x ± SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>8 weeks</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cholesterol (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>5.73 ± 0.29</td>
<td>5.76 ± 0.26</td>
<td>+ 0.5</td>
</tr>
<tr>
<td>D-003 5 mg/day</td>
<td>5.75 ± 0.28</td>
<td>5.16 ± 0.11</td>
<td><strong>+++++</strong> -10.0</td>
</tr>
<tr>
<td>D-003 10 mg/day</td>
<td>5.70 ± 0.26</td>
<td>4.99 ± 0.12</td>
<td><strong>+++++</strong> -12.5</td>
</tr>
<tr>
<td><strong>LDL-C (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>4.02 ± 0.22</td>
<td>4.03 ± 0.19</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>D-003 5 mg/day</td>
<td>4.04 ± 0.33</td>
<td>3.48 ± 0.15</td>
<td><strong>++</strong> -13.9</td>
</tr>
<tr>
<td>D-003 10 mg/day</td>
<td>3.89 ± 0.26</td>
<td>3.04 ± 0.14</td>
<td><strong>++</strong> -21.8</td>
</tr>
<tr>
<td><strong>HDL-C (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1.08 ± 0.18</td>
<td>1.04 ± 0.13</td>
<td>- 3.7</td>
</tr>
<tr>
<td>D-003 5 mg/day</td>
<td>1.05 ± 0.18</td>
<td>1.11 ± 0.13</td>
<td>* <strong>5.7</strong></td>
</tr>
<tr>
<td>D-003 10 mg/day</td>
<td>1.13 ± 0.21</td>
<td>1.34 ± 0.16</td>
<td><strong>++</strong> +18.5</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>1.71 ± 0.63</td>
<td>1.84 ± 0.49</td>
<td>+ 5.8</td>
</tr>
<tr>
<td>D-003 5 mg/day</td>
<td>1.81 ± 0.72</td>
<td>1.69 ± 0.38</td>
<td>- 6.6</td>
</tr>
<tr>
<td>D-003 10 mg/day</td>
<td>1.83 ± 0.55</td>
<td>1.63 ± 0.32</td>
<td>* - 10.9</td>
</tr>
</tbody>
</table>

* p < 0.05; **p < 0.001 Comparison with baseline (Wilcoxon test for paired samples)
+ p < 0.05; ++ p < 0.0001 Comparison with placebo (Mann Whitney U test)

**DISCUSSION**

The present study demonstrates that D-003 administered at 5 and 10 mg/day for 8 weeks to older subjects inhibits the markers of LP here assessed. At both doses, D-003 protected LDL particles against LP, as supported the increase on lag phase and decreased propagation rate of the diene generation time course of LDL copper dependent LP. These results are in line with previous experimental and clinical data. The “in vitro” generation of conjugated dienes by copper appears to be a good surrogate of oxidative stress in humans and correlated well with “in vivo” LDL oxidation estimation, since oxidation by copper ions partially mimics “in vivo” situation. In particular, there are evidences supporting a relationship between the susceptibility of LDL to “in vitro” oxidation and atherogenic risk. Besides, aging process may enhance oxidative stress, which renders an increased susceptibility of LDL to oxidation in the elderly.

Considering the available evidence of a strong association between the oxidative modification of LDL and atherosclerosis development, preventive effects of D-003 on LP of LDL particles could be considered as potentially beneficial for the antiatherogenic profile of the drug. Beyond the effects on LP of LDL molecule, D-003 5 and 10 mg/day also significantly increased TAS, which indicates that the treatment increases total radical-antioxidant potential of plasma, suggesting the possibility of an augmentation of water and non-water soluble antioxidant capacity of plasma after D-003 treatment. Also, D-003 at 10 mg/day, but not at the lowest dose tested (5 mg/day) decreased MDA concentration, determined throughout TBARS levels. Considering that MDA is a final product formed by decomposition of lipid to LP products, such result seems contradictory with those above referred. This apparent contradiction could be related with the fact that assessment of MDA throughout TBARS measurement in a complex biological sample such as plasma is not specific enough, so that other compounds could interfere the results. Then, further studies need to investigate the effects of D-003 on MDA production by using direct methods of MDA estimation.

As expected, D-003 (5 and 10 mg/day) showed cholesterol-lowering effects, reducing LDL-C and CT, whereas increasing HDL-C. D-003 at 10 mg/day modestly reduced triglycerides too. The effects here reported generally agrees with previous results of the effects of D-003 on lipid profile, except TC reduction, that was below the expected percent reductions, a fact without conclusive explanation.

Thus, the fact that inhibition of LP of LDL particles and the effect on the other LP markers here assessed have been obtained with the same doses of D-003 effective for lowering LDL-C and TC, while increasing HDL-C represents an advantage for the expected antiatherogenic effects of the treatment. To estimate the potential benefit derived from these results, first we need to recognize that we actually demonstrated is that D-003 protect oxidation of LDL to copper-induced oxidation “in vitro” in a free-cell system, which
includes only factors intrinsic to LDL. The susceptibility of LDL to oxidation “in vivo”, however, is also influenced by the extrinsic factors due to the microenvironment where LDL is found, but protective effects of HDL-C have been mostly associated to the ability of HDL for removing the excess of cholesterol from peripheral tissue throughout the reverse cholesterol transport. Nevertheless, recent works have suggested the ability of HDL to protect LDL against oxidation, by the action of two HDL-associated enzymes, paraoxonase and arylesterases could contribute to such protective effects. Besides, HDL can reduce hydroperoxides to their corresponding hydroxides, which could contribute to minimize the deleterious effect of LP on LDL.

Among the potential benefits of D-003 treatment, moderate to marked and fast increases on HDL-C levels have been reported, the present results being consistent with such appreciation. Thus, the increase of HDL observed in the present work may be important, bearing in mind that HDL is present in the artery interstitial space at a concentration much higher than LDL, which could contribute to achieve “in vivo” protection of LDL by the above mentioned mechanisms.

Consistently with previous results, D-003 was well tolerated. No-drug related disturbances on safety indicators were found. No patient withdrew from the study and only three (2 placebo, 1 D-003 5 mg/day) reported mild AE.

CONCLUSIONS

D-003 (5 and 10 mg/d) administered for 8 weeks to older subjects effectively prevented LP of LDL, increasing lag t and lowering Vmax of conjugated. Also, D-003 increased TAS and reduced TBARS levels, but effects were different from placebo only for 10 mg/d. D-003 did not modify the activity of the antioxidant enzymes assessed. As expected, D-003 induced cholesterol-lowering effects characterized by reductions of LDL-C, TC and increases on HDL-C levels, but a modest reduction of triglycerides was also obtained. The present results add new data on the antioxidant effects of D-003, including demonstration of preventive effects on LP marker in the elderly, a population subset wherein oxidative processes are increased. Nevertheless, the extent of the effects of D-003 on LP processes deserves further investigation including studies with larger sample sizes, doses and treatment duration.

REFERENCES


