Effects of Lovastatin and Pravastatin on ubiquinone and 4-hydroxynonenal tissue levels in the hypercholesterolemic hamster

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ABSTRACT

Further to our experiment which suggested that lovastatin treatment leads to a larger decrease of ubiquinone levels in rat tissues than pravastatin, we undertook to confirm this finding in the hypercholesterolemic hamster, a model that mimics the cholesterol regulatory mechanisms in humans. In view of the proposed antioxidant role of ubiquinone, we also tested the hypothesis that ubiquinone level decreases would be associated with an increase in peroxidation products, i.e. 4-hydroxynonenal (HNE), and one of its major metabolite, 1,4-dihydroxynonene (DHN) in the myocardium. Methods: Lovastatin and pravastatin were administered at a dose of 20 mg/kg/day for 30 days to male Golden Syrian hamsters fed a hypercholesterolemic diet. Results: In blood, myocardium and skeletal muscle decreases in Q₉ levels were observed with both drugs (29% to 42%, p<0.05). In the liver, no effects were observed in Q₉ levels vs. control while the difference between the treatment groups was significant. No differences were seen in ubiquinone-10 (Q₁₀) levels in the blood, myocardium, or skeletal muscle. In the liver, significant decrease vs. control (21% for lovastatin and 14% for pravastatin, p<0.05) were observed in Q₁₀ levels. For lovastatin and pravastatin similar decreases in protein-bound HNE (15% and 35% respectively, p<0.05 overall) and DHN (21% and 25% respectively, p<0.05 overall) were observed in myocardium. Conclusions: Our results support previous reports indicating that HMG-CoA reductase inhibitors decrease ubiquinone tissue levels. However, this decrease was similar with lovastatin and pravastatin contrary to what was observed in the rat. The HNE results do not confirm our hypothesis on the antioxidant activity of ubiquinone in the myocardium but rather suggest for lovastatin and pravastatin, an additional preventive effect on the progression of atherosclerotic.
INTRODUCTION

Ubiquinone is a lipid soluble product endogenously synthesized by means of the mevalonate pathway. In a previous experiment, we elected to compare the effects of two model drugs, hydrophilic pravastatin and lipophilic lovastatin, on ubiquinone levels [1] in view of their different tissue selectivity [2-4]. We observed a decrease in ubiquinone tissue levels in rats treated with HMG-CoA reductase inhibitors for 4 weeks [1] which confirmed previous findings [5-8]. This decrease could theoretically be linked to some of the adverse effects reported with HMG-CoA reductase inhibitors such as myopathy [9,10]. *In vitro* data suggest that lovastatin is more myotoxic than pravastatin [11-12]. In our study the decrease in the ubiquinone myocardium levels was more pronounced in rats treated with lovastatin than those treated with pravastatin and could probably be attributed to a greater uptake of lovastatin by the myocardium owing to it’s greater lipid solubility and tissue affinity.

However, treatment with lovastatin and pravastatin did not induce any significant changes in the lipid levels [1]. This finding was consistent with previous reports [13-15] and was attributed to an exaggerated response of the rats with regard to cholesterol biosynthesis (further to treatment by HMG-CoA reductase inhibitors). Indeed the rat has an exceptionally high capacity to modulate the absolute rates of cholesterol synthesis and degradation, and responds quickly to cholesterol imbalances by promoting/inhibiting one of these processes [16]. Since modulation of LDL-C (Low Density Lipoprotein Cholesterol) transport activity is rarely needed in this species, plasma LDL-C levels remain remarkably constant under a variety of dietary and pharmacological challenges [16]. Because of this strong ability to compensate for the inhibition of cholesterol synthesis by HMG-CoA reductase inhibitors, it was desirable to reproduce this experiment in a species devoid of that attribute.

The species that best mimics the human response to HMG-CoA reductase inhibitors is the hamster [17-22]. It has proven to be a good animal model for the study of cholesterol regulatory mechanisms (such as the rate of receptor-dependent LDL-C degradation and the rate of synthesis of LDL-C particles). In the male hamster, the concentration of plasma LDL-C responds to cholesterol imbalances in a manner that is similar to that seen in man [17-19]. Furthermore, the kinetics of LDL-C production and degradation have been described in detail in this species [17]. The hamsters have been shown to be good responders after hypocholesterolemic treatment with HMG-CoA reductase inhibitors [20-22].

Ubiquinone (in its reduced form, ubiquinol) has been shown to act as an antioxidant which helps the tissue to maintain its capacity to withstand an oxidative stress [23-28]. Ubiquinol is the only known lipid-soluble antioxidant that animal cells can synthesize *de novo* and for which a powerful enzymatic mechanism exists, the interconversion ubiquinone/ubiquinol, which can regenerate ubiquinol from the ubisemiquinone [29]. Ubiquinol acts primarily by preventing the formation of lipid peroxyl radicals further to reducing the initiating perferryl radical [23]. In addition, ubiquinol may act by eliminating lipid peroxyl radicals either directly or through the regeneration of tocopherol from the tocopheroxy radical i.e.
ubiquinol may prevent both the initiation and the propagation of lipid peroxidation [23]. Therefore, it appeared reasonable to hypothesize that decreases in tissue ubiquinone levels could lead to an increase in the peroxidation products.

Lipid peroxidation often occurs in response to oxidative stress, and a great diversity of aldehydes are formed when lipid hydroperoxides break down in biological systems [30]. Unlike free radicals, aldehydes are rather long lived and can therefore diffuse from the site of their origin (i.e. membranes) and reach and attack targets intracellularly or extracellularly which are distant from the initial free radical event. To evaluate the effects on lipid peroxidation a number of techniques are available among which the thiobarbituric acid method. However, this technique lacks selectivity. We have elected to measure 4-hydroxyhexenal (HNE) as a marker of lipid peroxidation because among the lipid peroxidation products it is the most cytotoxic product endogenously generated [30]. We wanted to measure HNE levels in the myocardium since it is more prone to oxidant activity and because HNE has been shown to induce cardiomyocyte damage [31]. A decreased capacity to withstand oxidative stress could partially explain the pathological conditions associated with decreased ubiquinone levels in the myocardium [32].

The primary objective of the present study was to document the effects of lovastatin and pravastatin on ubiquinone and HNE levels in the hypercholesterolemic hamster. We also wanted to document the interrelationships between the effects of lovastatin or pravastatin on the ubiquinone and lipid levels.

**MATERIALS AND METHODS**

This study was approved by the animal ethics committee of the University of Montreal, Quebec, Canada where it was conducted.

**Study Design**

Three groups of 18 male Golden Syrian hamsters were assigned to treatment with either pravastatin or lovastatin or to a control group. The hamsters received once daily, by gavage, a 20 mg/kg dose of either drug in the vehicle or of the vehicle only for 30 days. The weight of the hamsters was recorded at the start of the treatment period and twice per week thereafter.

**Animals and diet:** Male Golden Syrian hamsters (Charles River Breeding Laboratories Inc., Kingston, NY) weighing 80-100 g were housed in colony cages and subjected daily to 12h of light (19:00 to 07:00 hr) and 12h of dark (07:00 to 19:00 hr) during the acclimatization period (4 days with a normal diet followed by 6 days with the hypercholesterolemic diet) and throughout the experiments. The basic diet used in this experiment was Rodent Laboratory Chow 5001 (Ralston Purina Canada Inc., LaSalle, Qc) containing 0.34 mg/g of cholesterol and 49 mg/g of total fat. Triacylglycerol (copra oil) was added to the diets at a concentration of 20% so that 20 g of copra oil was mixed with 80 g of the ground chow. Cholesterol was added to the diet at a concentration of 0.12%, which corresponds to 1.2 mg of added cholesterol per gram of food. The cholesterol was solubilized in warm ethanol and added to the diet while mixing in a mechanical food blender to evaporate the ethanol. The diets were fed ad lib and experiments were carried out during the mid-dark phase of the light cycle.
Drugs: Pentobarbital was purchased from MTC Pharmaceuticals (Cambridge, ON, Canada). Lovastatin was provided by Merck and Co. Inc. (Rahway, NJ, USA) and pravastatin was provided by Bristol Myers Squibb (Princeton, NJ, USA). The HMG-CoA reductase inhibitors were dispensed as a 2 mg/mL suspension of lovastatin or pravastatin mixed in a freshly-prepared 1% carboxymethylcellulose solution. Each animal received 1 mL of suspension per 100 mg body weight (20 mg/kg/day) by oral gavage. The control group received an equal volume of the vehicle. HMG-CoA reductase inhibitors were given for a period of 30 days as lovastatin was previously shown to cause a maximal decrease in plasma cholesterol levels in hamsters within a 12 to 14-day period [21, 33]. This dose, which is over 50-fold the therapeutic dose in humans, was selected in view of its reported hypocholesterolemic effect in hamsters [21, 33].

Samples Handling: After the 30-day treatment, the hamsters were euthanized with pentobarbital and samples of heart, skeletal muscle, liver and blood were immediately frozen ("freeze clamped" at -196°C), kept in liquid nitrogen until long-term storage in aliquots at -80°C. The tissues were accurately weighed (=100 mg) in the frozen state and homogenized with 1 mL of 1 mM buffer solution (NaH$_2$PO$_4$•12H$_2$O and Na$_2$HPO$_4$•7H$_2$O adjusted to pH 7.4) in a homogenizer with a motor-driven pestle (a Potter-Elvehjem homogenizer, Polytron, Kinematica GMBH, Luzern, Switzerland). A volume of 50 µL of a solution of butylated hydroxytoluene (BHT) in reagent alcohol (10 mg/mL) was added to each sample to prevent autooxidation [34]. For the blood, 0.2 mL samples of whole blood were mixed with 0.8 mL of 0.1 M sodium dodecylsulfate (SDS). Then 1.0 mL of 5 mM ascorbate in 5 mM phosphate buffer (pH 7.4) was added and the mixture was vortex-mixed for 1 min.

ANALYSES

Lipid Analyses: Lipid analyses were performed on blood plasma samples by the Cirion Diagnostic Investigation Group (Laval, Canada), using the standard clinical chemistry method. For LDL-C, a comparison between the standard method (i.e. using the Friedewald formula: LDL-C=[total-C]-[HDL-C]-[TG/2.22], where HDL-C represents the High Density Lipoprotein Cholesterol and TG, the triglycerides, and a direct quantitative measurement method [35-37] was also performed.

Analysis of Ubiquinones: Tissue ubiquinone levels (in its oxidized form) were determined using a procedure which is described in detail elsewhere [1, 38]. Chemicals and Reagents: Solvents of HPLC grade and all other chemicals of analytical grade were purchased from Anachemia (Montreal, Canada). The internal standard ubiquinone-11 (Q$_{11}$), was kindly provided by the Nisshin Chemicals Company, Tokyo, Japan. Ubiquinone-9 (Q$_9$) and ubiquinone-10 (Q$_{10}$), BHT, triacylglycerol, cholesterol, carboxymethylcellulose, and ascorbic acid were obtained from Sigma (St. Louis, MO, USA). SDS was obtained from BDH Biochemicals (Toronto, Canada). Instrumentation: A Speed-vac plus concentrator (Model SC-210A, SAVANT Instruments, Farmingdale, NY, USA), and a Speed Mate 10 vacuum manifold (Applied Separations, Lehigh Valley, PA, USA) were used. The HPLC system consisted of two Constametric III pumps (LDC Milton Roy, LDC Division, Riviera Beach, Fla,USA), a model 7125 injector (Rheodyne, Cotati, CA), a UV detector (Waters, Milford, MA) set at a wavelength of 275 nm and an integrator (Shimadzu
Chromatopac CR 601, Kyoto, Japan). The octadecyl silica (ODS) column (12.5 cm x 4.6 mm) was packed with 5 µm inner diameter Spherisorb C8 (Hichrom, Reading, U.K.).

**Method:** Briefly, after addition of SDS and reagent alcohol the sample was further vortexed for 1 minute. The ubiquinone was extracted by liquid-liquid extraction with hexane (2 x 4 mL) and subsequently redissolved in methanol: ethanol (70:30 v/v), the mobile phase. All extraction steps were performed in the absence of incandescent light or direct sunlight. The samples were injected in the HPLC shortly after reconstitution. The UV detection was performed at 275 nm, the maximum absorbance for the ubiquinone. The ubiquinone levels in control tissues were within the ranges of those previously reported [39].

**Analyses of 4-hydroxy-trans-2nonenal (HNE) and its metabolite, 1,4-dihydroxy-2-none (DHN)**

This method is based on a previously reported isotope dilution gas chromatographic-mass spectrometric (GCMS) assay [40], which was modified to include a treatment with Raney Nickel to quantify protein-bound HNE and DHN levels. **Chemicals and Reagents:** Sodium borodeuteride (NaB$_2$H$_4$) and (5,5,6,6,7,7,8,8,9,9,9-^{2}$H$_{11}$) HNE diethyl acetal (^{2}$H$_{11}$-HNE diethyl acetal) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and CDM isotopes (Pointe-Claire, QC, Canada), respectively. Chemicals, organic solvents and acids were obtained from Laboratoire Mat (Montreal, QC, Canada), Sigma (St-Louis, MO, USA), J. T. Baker (Phillipsburg, NJ, U.S.A.), Boehringer Mannheim GmbH (Germany) and Fisher Scientific (Fair Lawn, NJ, U.S.A.). The derivatization agent, N-methyl-N-(t-butyldimethyltrifluoroacetamide) was obtained from Regis Chemical (Morton Grove, IL, USA). All aqueous solutions were made with water purified by the Elgastat Maxima UF system (England). The stock solution of the deuterated internal standard of (^{2}$H$_{11}$) DHN (1 mM) was prepared as follows: (i) hydrolysis of the (^{2}$H$_{11}$) HNE diethyl acetal to (^{2}$H$_{11}$) HNE in diluted citric acid (1%), (ii) spectrophotometric determination of the concentration of [^{3}$H$_{11}$] HNE and (iii) treatment with NABH$_4$ to reduce (^{2}$H$_{11}$) HNE to (^{2}$H$_{11}$) DHN. The stock solution of (^{2}$H$_{11}$) DHN was kept at –80 °C. **Instrumentation:** The GCMS system was a Hewlett-Packard 5890 Series II plus gas chromatograph coupled to a 5989B mass spectrometer. This instrument was equipped with a HP 5 capillary column (25 m x 0.2 mm inner diameter x 0.33 µm film thickness). The mass spectrometer was operated in the positive chemical ionization mode after calibration with an automatic electron impact and a manual positive chemical ionization mode (ammonia pressure of 7.2 x 10$^{-1}$ Torr and electron energy of 153 + eV) in order to optimize sensitivity in the higher mass range. Split ratio injection was about 10/1, carrier gas helium was 0.7 mL/min, injection port 280°C, transfer line 290°C and column head pressure at 23 psi. Appropriate ion sets were monitored with a dwell time of 100 ms per ion. The column temperature was initially set at 150°C for 2 min, increased by 5°C/min to 215°C and then by 30°C to 280°C. **Method:** The homogenized heart samples were treated with NaB$_3$H$_4$ 1M (250 µL), slightly vortexed and then left on ice for 30 min. This treatment reduces HNE to chemically stable (1-^{2}H) DHN. Then, a saturated sulfosalicylic acid solution (250 µL) was added to the heart homogenates and proteins were separated by centrifugation at 12000 rpm for 15 min at 4°C. The proteins were then incubated with 2 mL of guanidine solution (8 M), 1 mL of (^{2}$H$_{11}$)-DHN (1 µM)
and 2.29 g of Raney Nickel [41] catalyst for 15 hours at 56°C, followed by sequential centrifugation for 5 min at 2000 rpm at room temperature and for 12 min at 2000 rpm at 4°C. Note that treatment with Raney Nickel converts DHN, (1-2H) DHN and (2H11) DHN to their corresponding saturated derivatives (1,4-dihydroxynonanal). The supernatant was brought to pH <2 and extracted twice with 5 mL anhydrous ether, by vortexing for less than 20 sec. The combined ether extracts were evaporated under N2 to a residual volume of about 5 µL and the residue was treated with 75 µL methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide. Samples were left overnight under a hood at 70 °C. A 1 µL sample was injected into the GCMS. The following two ion sets were monitored for the analysis of DHN, HNE and the internal standard (2H11) DHN: (i) 257, 258 and 268 and (ii) 389, 390 and 400. The first ion set, the most abundant in the ammonia chemical ionization mass spectrum, probably results from the loss of (-TBDMS), whereas the second one corresponds to the (M + H)+ ions of the di-tert-butyldimethylsilyl (di-TBDMS) derivatives of 1,4-hydroxynonane (257, 389), 4-hydroxynonanal (258, 390) and (2H11)-1,4-dihydroxynonane (268, 400). Calculations: Calculations of the quantities of HNE (QHNE) and DHN (QDHN) in samples were previously described in detail [40]. Briefly, this involved introducing the areas, corrected for natural abundance, of the ions corresponding to the analyzed compounds (CAHNE or CADHN or CAIS) and the quantity of the internal standard (QIS) added to each sample analyzed:

\[ Q_{\text{HNE}} = Q_{\text{IS}} \times \left( \frac{C_{\text{HNE}}}{C_{\text{IS}}} \right) \quad \text{or} \quad Q_{\text{DHN}} = Q_{\text{IS}} \times \left( \frac{C_{\text{DHN}}}{C_{\text{IS}}} \right). \]

Quantities of DHN and HNE, calculated separately for the two different ions sets for each injection, did not differ significantly and were thus averaged. Quantities of DHN and HNE reported in this study represent the average of duplicate or triplicate injections for which the coefficient of variations was 7.69 ± 0.75% for all ions monitored.

Statistics

The results are expressed as means ± SD. Ubiquinone, HNE and DHN levels were tested for overall treatment effect (placebo vs. lovastatin vs. pravastatin) by ANOVA (α=0.05). Pairs of treatments were compared with Bonferroni adjustments for multiple comparisons. Lipids were compared across groups using a General Linear Model. Linear regression analysis was used to compare the direct LDL-C method [35] with the Friedewald equation for: animals with triglyceride levels <4.5 mM. Differences between LDL-C levels using the direct LDL-C assay and the Friedewald equation were analyzed by paired t-test (α=0.05). Weights were compared across groups using a General Linear Model to test for treatment and time x treatment interactions.

RESULTS

Weight Changes

Treatment of hamsters fed a hypercholesterolemic diet with either the vehicle, lovastatin or pravastatin for 30 days did not result in a difference in weight changes. Further to the General Linear Model analysis neither time nor time x treatment were significant.

Lipid levels (Table I)
The effects of a 30-day pretreatment with lovastatin and pravastatin on total cholesterol, HDL-C and triglycerides are shown in Table I. No effects on total cholesterol were observed following treatment with either lovastatin or pravastatin. Decreases in triglycerides vs. the control group were observed: 37.7% for lovastatin (p=0.0001) and 59.0% for pravastatin (p=0.0001). A similar increase in HDL-C levels was observed for both lovastatin (16.4%, p=0.0381) and pravastatin (16.9%, p=0.0357). The LDL-C results, obtained using a direct quantitative measurement method [35-37] were further compared to the results obtained using the Friedewald formula. (a) Regression: There was a good correlation between the two methods: for animals with triglyceride levels < 4.5 mM: \( r^2 = 0.83 \), (b) T-Test: Overall the LDL-C results obtained with the direct method were significantly greater than those obtained using the Friedewald equation for all animals with triglyceride levels < 4.5 mM (3.86 ± 1.24 mM vs. 3.38 ± 1.68 mM, p=0.003). An increase in LDL-C levels was observed in the pravastatin-treated group (42.1%, p=0.0038). This was also greater than the 12.3% increase observed in the lovastatin group (p=0.04). However, no differences in LDL-C levels were observed in the lovastatin group compared to the control group.

Table I: *Plasma lipid levels*: vehicle, pravastatin and lovastatin administered at a dose of 20 mg/kg/day for 30 days.

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mM)</th>
<th>TG (mM)</th>
<th>HDL-C (mM)</th>
<th>LDL-C (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>6.99</td>
<td>5.95*</td>
<td>1.95</td>
<td>3.09</td>
</tr>
<tr>
<td>SD</td>
<td>1.45</td>
<td>1.8</td>
<td>0.44</td>
<td>0.75</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>16</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td><strong>Lovastatin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.47</td>
<td>3.71a</td>
<td>2.27a</td>
<td>3.47a,b</td>
</tr>
<tr>
<td>SD</td>
<td>1.63</td>
<td>1.23</td>
<td>0.35</td>
<td>1.10</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>18</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td><strong>Pravastatin</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>7.76</td>
<td>2.44a</td>
<td>2.28a</td>
<td>4.39a,b</td>
</tr>
<tr>
<td>SD</td>
<td>1.69</td>
<td>0.81</td>
<td>0.37</td>
<td>1.05</td>
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<tr>
<td>n</td>
<td>14</td>
<td>18</td>
<td>18</td>
<td>16</td>
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The “a” represents a p-value of <0.05 when compared to the control group and the “b” represents a p-value of <0.05 when compared to the other HMG-CoA reductase inhibitor treated group. The triglyceride values marked with an asterisk represent a value that is too high for the Friedewald formula to be acceptable for the calculation of the LDL-C levels.

**Ubiquinone levels (Figure 1)**

Four tissues (blood, myocardium, skeletal muscle and liver) were analyzed for their contents in ubiquinone-9 and -10 and the results were as follows:

**Ubiquinone-9:**

*Myocardium (figure 1a):* Decreases of 30% in Q9 levels for lovastatin and 29% for pravastatin. In both treatment groups decreases were significant versus the control group.
(p<0.05). The overall treatment effect was significant (p=0.0001). **Skeletal Muscle (figure 1b):** Decreases of 30% in Q₉ levels for lovastatin and 29% for pravastatin. In both treatment groups decreases were significant versus the control group (p<0.05). The overall treatment effect was significant (p=0.0001). **Blood (figure 1c):** Decreases of 42% in Q₉ levels for lovastatin and 33% for pravastatin. In both treatment groups decreases were significant versus the control group (p<0.05). The overall treatment effect was significant (p=0.005). There was no difference between the treated groups for either the myocardium, skeletal muscle, or blood. **Liver (figure 1d):** No change in Q₉ levels was observed with either lovastatin or pravastatin when compared to the control group. Despite the lack of overall treatment effect, decreases with lovastatin (10%) were significantly different than increases with pravastatin (9%) (p<0.05).
Control Lovastatin Pravastatin

ug/g
Q9 or Q10

20/mg/kg/day 20/mg/kg/day

Q9 or Q10

85 ± 13
(n=13)

60 ± 11
(n=14)

61 ± 11
(n=15)

23 ± 5
(n=13)

21 ± 4
(n=14)

22 ± 4
(n=15)

ug/mL
Q9 or Q10

Control Lovastatin Pravastatin

20/mg/kg/day 20/mg/kg/day

Q9 or Q10

0.12 ± 0.04
(n=11)

0.12 ± 0.04
(n=13)

0.07 ± 0.04
(n=14)

0.10 ± 0.03
(n=17)

0.10 ± 0.04
(n=16)

0.10 ± 0.04
(n=15)
Figure 1: Ubiquinone-9 and-10 tissue levels after a 30-day treatment with either pravastatin, lovastatin or vehicle: a) myocardium, b) skeletal muscle, c) blood and d) liver. The “a” represents a p-value of <0.05 when compared to the control group and the “b” represents a p-value of <0.05 when compared to the other HMG-CoA reductase inhibitor treated group.

Ubiquinone-10:
No difference was observed in Q₁₀ levels, in the myocardium, skeletal muscle and blood, but the decreases with both lovastatin and pravastatin showed a similar trend. In the liver, decreases in Q₁₀ levels were observed with both lovastatin (21%) and pravastatin (14%). These decreases were significant versus the control group (p<0.05). The overall treatment effect was significant (p=0.0008). There was no difference between the treated groups.

4-Hydroxynonenal levels in the myocardium
Myocardial tissue samples were analyzed for their HNE and DHN content with the following results (figure 2): Decreases in protein-bound HNE myocardium levels of 15% for lovastatin and 35% for pravastatin and in DHN myocardium levels, of 21% for lovastatin and 25% for pravastatin. The overall treatment effect was significant for both HNE (p=0.049) and for DHN (p=0.007). A comparison of treatment pairs showed no difference between lovastatin and pravastatin for either protein-bound HNE or DHN. However, only the pravastatin group, for DHN, was significantly different from the control group.
**Figure 2:** 4-hydroxynonenal and 1,4-dihydroxynonene myocardium levels after a 30-day treatment with either pravastatin, lovastatin or vehicle. The “a” represents a p-value of $0<0.05$ when compared to the control group.

**DISCUSSION**

The major findings of the study are the following: lovastatin and pravastatin decreased to a similar extent the ubiquinone levels in the blood, myocardium and skeletal muscle in our hypercholesterolemic hamster model. For both drugs, decreases in ubiquinone levels were associated with decreases in HNE and DHN levels. These findings do not support our research hypothesis, i.e. that a decrease in ubiquinone levels would result in an increase in peroxidation products, but rather suggest a potential antioxidant role for lovastatin and pravastatin.

**Animal Model:** In the present study, the effects of lovastatin and pravastatin on plasma cholesterol levels were marginal, but the decreases in triglyceride and increases in HDL-C levels were significant. For LDL-C, an increase was observed in the pravastatin group. In order to interpret these data, one must consider that all animals were fed a hypercholesterolemic diet and were not fasted at the sampling time. The lipid levels are usually measured in fasted subjects (human or animal) [36]. Since no correlation would be expected between fasting and non-fasting animals [37], the lipid results in non-fasted subjects after a hypocholesterolemic treatment would not be expected to parallel those in fasted subjects. Thus, the nutritional state of the animals at sampling time could explain why our lipid results were considerably higher than those measured in fasted animals [33], although they are in line with those previously reported in non-fasted animals [42].
Moreover, the administration of cholesterol and triacylglycerol in the control group would be expected to increase the secretion of very low density lipoproteins which might partly account for the observed increases in LDL-C production [43]. Although, in our study, the difference between the increases in the LDL-C plasma levels in the pravastatin group and in the lovastatin group is not fully explained, we consider the following explanations to be unlikely. (i) \textit{LDL-C Analysis:} The direct LDL-C measurement is the method of choice in animals fed a hypercholesterolemic diet since the Friedewald equation is not accurate when TG levels are superior to 4.5 mM. In the control group, only two animals had triglyceride levels below that threshold. However, a correlation was established between these two methods in animals with triglyceride levels inferior to 4.5 mM. (ii) \textit{Differential tissue distribution or plasma levels of the drugs:} Lovastatin and pravastatin are distributed to the same extent in the liver [14], therefore the effect on LDL-C plasma levels (further to their effect on cholesterol biosynthesis and LDL-C receptors in the liver) should be similar. Further, the drug plasma levels, although not measured in this study, were previously shown not to be related to their hypocholesterolemic effect [44]. (iii) \textit{Diet:} Since there was no difference in weight changes between the pravastatin and lovastatin-treated animals, the difference in LDL-C levels cannot be attributed to the diet. Therefore, the differential effects of pravastatin on LDL-C remain to be explained.

\textbf{Effects on ubiquinone:} Results from our previous experiments in rats, indicated that lovastatin lowered myocardium concentrations of ubiquinone to a greater extent than pravastatin [1]. Previous investigations [2, 45] have shown that lovastatin is present at higher concentrations than pravastatin in the majority of organs with the exception of the liver and ileum. It is noteworthy that, in a recent clinical study, lovastatin led to greater decreases of ubiquinone serum levels than pravastatin and that the decreases were dose-dependent [7]. The present experiment do not support these findings since ubiquinone levels in blood, myocardium and skeletal muscle were found to decrease similarly with lovastatin and pravastatin. We do not have an explanation for these differences, although they may be related to the nutritional state of the animal (feeding of a high fat and cholesterol diet in this study, vs. a normal diet in our previous study) or to the species capacity for drug absorption or metabolism (rat vs. hamsters).

\textbf{Effects on HNE:} A similar decrease in myocardial protein-bound HNE and DHN levels was found for lovastatin and pravastatin. These results argue against our hypothesis that the decreased ubiquinone levels observed after treatment with these drugs would be associated with an increase in lipid peroxidation. Possible explanations for these data are: (i) the two drugs have antioxidant activities or (ii) ubiquinone has normally a pro-oxidant activity in the myocardium [46-47].

An antioxidant activity for pravastatin and lovastatin could compensate for the decrease in ubiquinone levels. Such an activity has been reported for pravastatin in human blood [48-49], for lovastatin in rabbit blood [50] and for fluvastatin in the blood of hypercholesterolemic patients [51]. Among possible explanations that have been suggested in support of an antioxidant role of HMG-CoA reductase inhibitors [50-54] the only one that applies to our experimental conditions is their chemical structure. The presence of H, CH$_3$, O and OH bonds (as well as the aromatic ring) could quench free radicals [50] such as hydroxyl and singlet oxygen which could initiate the lipid peroxidation [55]. However, some investigators have reported an opposite effect for lovastatin i.e. an enhanced potential
for oxidation under oxidative stress [56]. Recently it has been reported that the antioxidant activity of atorvastatin was due to one of its major metabolite (the \( \alpha \)-hydroxy) and not to the parent drug [57]. This could also be the case for lovastatin and pravastatin.

As for the possibility of a pro-oxidant activity of ubiquinone in the myocardium, it cannot be excluded [58] when one consider that (i) it is the reduced form (i.e. ubiquinol) which is responsible for the antioxidant activity and (ii) in the myocardium, the oxidized form (i.e. ubiquinone) is preponderant (> 90%). In contrast, in other organs such as the liver, the oxidized form represents only 60% of the total [59]. Therefore, it is possible that ubiquinone could only exert significant antioxidant activity in the tissues where the reduced form is preponderant [59-60]. However, our HNE and DHN data are not a definite evidence against the antioxidant activity of the ubiquinone/ubiquinol in the myocardium. It would be of interest to measure the HNE levels in a similar model, accentuating the response of the myocardium to an oxidative stress. This could be done either by ischemia-reperfusion episodes or after exercise training or in conditions where there is a greater need for ubiquinone synthesis.

**Conclusions:** Our results suggest that, in the hypercholesterolemic hamster, the decrease in ubiquinone levels in the myocardium, skeletal muscle and blood with lovastatin pre-treatment is similar to that observed with pravastatin. Further, the use of these drugs would not lead to an increase in peroxidation products in the myocardium. However, since our experimental conditions maintained baseline levels of redox potential, we cannot exclude the possibility that protein-bound HNE levels could be increased by an oxidative stress. In our study, the antioxidant activity of lovastatin and pravastatin, as evidenced by a decrease of protein-bound HNE and DHN content in the myocardium, suggests an additional preventive effect for these agents on the progression of atherosclerosis. This finding also underlines the importance of further investigating the ramifications associated with this potential effect.

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