EFFECT OF LOVASTATIN ON PLAQUE FORMATION AND LDL OXIDATION IN CHOLESTEROL-FED RABBITS

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ABSTRACT

The present study investigated the effect of lovastatin, a HMG-CoA reductase inhibitor, on the regression or progression of atherosclerosis and on the susceptibility of LDL to oxidation in vitro in cholesterol-fed rabbits. New Zealand White Rabbits were fed 1% cholesterol for 4 weeks, and 0.5% cholesterol for additional 12 weeks. One subgroup of cholesterol-fed rabbits was treated with lovastatin (10 mg/day) from week 5-16. By the method of Cu^{2+}-mediated LDL oxidation, oxidative susceptibility of LDL was measured at 4 and 16 weeks of the experimental period. Cholesterol-feeding induced a marked increase in oxidative susceptibility of LDL. Treatment with lovastatin had no effect on lag time, maximal diene production, or diene production rate, even though it reduced plasma total cholesterol concentrations by about 50%. At the time of sacrifice, the extent of atherosclerotic lesions was measured by morphometric analysis of intimal plaque area in the common carotid arteries. Carotid intimal plaque area was 15.8±4.6% after 4 weeks of cholesterol-feeding and 44.0±5.2% after 16 weeks. Lovastatin suppressed the progression of plaque formation (to 27.0±5.1%) but did not induce regression. These data suggest that lovastatin can slow the progression of atherosclerotic plaque formation in rabbits induced by cholesterol-feeding without decreasing the oxidative susceptibility of LDL.

Key words: intimal thickening, oxidized LDL, atherosclerosis

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INTRODUCTION

Clinical and experimental studies have established that elevated concentrations of low-density lipoprotein (LDL) are a risk factor for atherosclerosis[1-4]. The lipids deposited in the atherosclerotic lesions are mostly derived from plasma LDL, which is modified by oxidative processes, resulting in an enhanced uptake by the scavenger receptor of macrophages[4-6], leading to foam cell formation. Lipid peroxidation plays a critical role in atherosclerosis[7,8]. Oxidized LDL has been found to be chemotactic for monocytes[9]; it induces endothelial cell damage[10,11] and stimulates cytokine and growth factor release from cells of the arterial wall[4,7]. The severity of atherosclerosis has been found to correlate with the susceptibility of LDL to oxidation and the concentration of lipid peroxides[12]. The method of promotion of LDL oxidation by Cu^{2+} has provided an assay allowing to study the oxidizability of LDL ex vivo[13,14].

3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitors, like lovastatin, simvastatin, or pravastatin, are widely used to lower plasma LDL cholesterol concentrations. They are not only potent competitive inhibitors of the rate limiting enzyme in cholesterol biosynthesis[15,16], but also partly regulate LDL clearance by increasing the number of LDL receptors on the surface of liver cells[17,18]. There is increasing evidence that lovastatin can reduce atherosclerotic lesion development[19,20], as well as preserve endothelium-dependent relaxation both in hypercholesterolemic animal models and in humans[20-22]. In addition to reduced plasma cholesterol concentrations, the antiatherosclerotic activity of this class of compounds has been suggested to be due to reduced LDL oxidation[23-25]. However, this has not been supported by others[26].

To investigate the mechanism of antiatherosclerotic properties of HMG-CoA reductase inhibitors, the present experiment was designed to examine the effect of chronic treatment with lovastatin in cholesterol-fed rabbits with overt intimal lesions on the in vitro oxidation of LDL and the regression of atherosclerosis.

MATERIALS AND METHODS

Animals and study design

46 male New Zealand white rabbits weighing initially 1.5-2 kg were used in this study, which conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication. No 85-23, revised 1985) and had been approved by the Hannover supervisory committee for studies in animals. After a 2-week period of adaptation, the rabbits were exposed to dietary treatment for a period of 4 or 16 weeks. They were randomly divided into 4 groups. Rabbits in group I (n=12) were fed normal rabbit chow (Nohrlin 20 ZH5, Eggersmann, Germany) and plain tap water throughout the study and served as control group. The rabbits in group II (n=8) received 1% cholesterol diet (Altromin, Lage Germany) for 4 weeks (Cholesterol-4 group). The other two groups of eight rabbits were fed a diet containing 1% cholesterol for the first 4 weeks followed by 0.5% cholesterol (Cholesterol-16 group), or 0.5 % cholesterol supple-mented with 10 mg/day of lovastatin orally (MSD Sharp and Dohme, Germany; Lovastatin group) for the next 12 weeks. Animals were housed individually. Food and water were allowed ad libitum. Weight, food, and water consumption were measured in weekly intervals.

Blood samples were obtained from the central ear artery at baseline and at 4 and 16 weeks. Following

centrifugation (1500 x g, 10 min), plasma was removed and stored at -20°C prior to measuring plasma cholesterol. Another plasma sample was processed immidiately for the isolation and oxidation of LDL (see below).

At the end of 4 weeks, the rabbits in Cholesterol-4 group and 4 animals from the control group (Control-4 group) were sacrificed. The remaining rabbits were killed at 16 weeks. The carotid artery, thoracic and abdominal aortas were removed and cleaned of adherent fat and fascia.

Biochemical analyses

Plasma total cholesterol, LDL and HDL-cholesterol concentrations were quantified by commercially available enzymatic methods (Boehringer-Mannheim, Mannheim, Germany).

LDL isolation and oxidation

Fresh whole blood was collected into 1 mg EDTA/mL blood (Monovette-KE, Sarstedt, Germany) and centrifuged (1500 x g, 10 min) to generate plasma. LDL was isolated from plasma by high speed centrifugation through a discontinuous KBr gradient according to the method described by Esterbauer et al.[13]. Briefly, 5 mL of fresh plasma was adjusted to a density of 1.006 with KBr and centrifuged at 436,000 x g at 4°C for 2.5 hr using a Beckman TL-100 ultracentrifuge equipped with a Beckman TLA 100-2 Rotor (Beckman Instrument, U.S.A.). The VLDL fraction was collected, and the remaining solution was adjusted to a density of 1.063 with KBr. The LDL fraction was isolated by centrifugation at 436,000 x g for 2.5 hr at 4°C. EDTA (1 mg/mL) was present throughout all preparation steps. The EDTA-containing LDL stock solution could be stored overnight at 4°C in the dark in a nitrogen atmosphere. Before the susceptibility of LDL to oxidation was determined, the LDL stock solution was freed from EDTA by filtration through a disposable desalting column Econo-Pac 10 DG (Bio-Rad, Munich, Germany), using phosphate buffered saline solution (PBS) as eluent. Protein content was determined by the method of Bradford[27] using bovine serum albumin (BSA) as a standard.

LDL was adjusted with PBS to a concentration of 75 µg LDL protein/mL. Oxidation was triggered by adding 2.5 µM $CuSO_4$ to the LDL solution. Diene conjugation was monitored at 234 nm in 10 minute intervals using a Hitachi U-2000 spectrophotometer (Hitachi, Germany) for 6 hours. The concentration of conjugated dienes was calculated using the extinction coefficient of 2.8 x 10^4 $M^{-1}cm^{-1}$[13].

Using this method, LDL oxidation can be divided into three phases[13] : lag phase, propagation phase and decomposition phase which together describe the oxidizability of that particular LDL-preparation. The lag phase is defined as the period during which no oxidation occurs, i. e., the interval between the addition of $CuSO_4$ and the beginning of extensive lipid oxidation. The propagation phase occurs once LDL is depleted of its antioxidants and fatty acids are rapidly oxidized to form conjugated dienes, during which the rate of diene production can be calculated from the slope of the absorbance curve and expressed as micromoles of diene produced per minute per mg of LDL-protein. The levels of conjugated dienes reach a maximum and subsequently fall during the decomposition phase. The maximal diene production serves as one parameter, expressed as micromoles per mg of LDL-protein.

Morphometric and histological analyses

The proximal common carotid arteries were dissected free of adventitial tissue and cleared of remaining blood by infusion of 0.9% NaCl. Thereafter the arteries were opened longitudinally and placed on an even surface for photography of intimal lesions. Photographs were digitalized and measurements of total intimal area and plaque area were made by planimetry of the photographic images. The plaque areas were expressed as percentage of total intimal surface area.

Segments of the thoracic and abdominal aorta were excised, fixed in formalin, embedded in paraffin, and stained with hematoxylin/eosin for the morphometric measurement of intimal and medial cross-sectional areas by planimetry[28]. Four sections from each animal were analysed, and the values were averaged.

All histological measurements were performed by skilled observers who were blinded to the treatment groups.

Statistical analysis

All values are given as means ± standard error of the mean (S.E.M.). Statistical analysis was performed with the use of one way analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test. Correlations were performed by linear regression analysis. A p value of less than 0.05 was considered significant.

Table 1 Body weight, plasma total cholesterol and lipoprotein cholesterol concentrations of the rabbits on various groups.

	Control-4	cholesterol-4	control-16	cholesterol-16	chol.+lovastatin
Body weight (kg)					
baseline	2.7±0.07	2.66±0.66	2.78±0.05	2.76±0.06	2.70±0.08
4 weeks	3.21±0.04	3.25±0.14	3.29±0.06	3.29±0.07	3.40±0.07
16 weeks	-	-	4.21±0.09	3.66±0.11*	3.73±0.12*
Total cholesterol (mmol/L)					
baseline	0.73±0.03	1.06±0.11	1.12±0.07	1.21±0.06	1.23±0.09
4 weeks	0.66±0.06	41.14±3.78*	0.92±0.11	49.44±4.66*	46.28±6.20*
16 weeks	-	-	0.49±0.07	37.10±3.06*	23.38±1.34*#
LDL- cholesterol (mmol/L)					
baseline	0.12±0.02	0.17±0.04	0.28±0.04	0.26±0.02	0.34±0.05
4 weeks	0.07±0.02	39.20±3.69*	0.18±0.06	44.74±4.26*	41.72±5.74*
16 weeks	-	-	0.05±0.01	33.18±3.09*	20.75±0.64*#
HDL- cholesterol (mmol/L)					
baseline	0.42±0.02	0.56±0.05	0.56±0.04	0.55±0.02	0.57±0.04
4 weeks	0.33±0.05	0.35±0.03	0.46±0.06	0.40±0.04	0.47±0.02
16 weeks	-	-	0.21±0.03	0.22±0.03	0.21±0.02

All values are mean + S.E.M.

* P<0.05 VS control group at the same period

# P<0.05 VS cholesterol group at the same period

RESULTS

Body weight

The changes in body weights during the study period in all groups of rabbits are summarized in Table 1. There was a progressive increase in body weight with no significant differences between all experimental groups during the first 4 weeks. Thereafter the rabbits in the control group grew faster than those rabbits fed a cholesterol-enriched diet, and showed significantly higher body weights than the cholesterol-fed rabbits at 16 weeks ($P<0.05$). No significant difference in body weights was found between the cholesterol-16 and lovastatin group.

Plasma lipids and lipoproteins

The effects of the cholesterol diets and treatment with lovastatin on plasma total cholesterol and lipoprotein concentrations are shown in Table 1. Total cholesterol was 1.15 ± 0.03 mmol/L (n = 36) at baseline with no significant differences between the groups. It remained unchanged during the whole experimental period in the control-4 and control-16 groups. Plasma total cholesterol concentrations increased to 46.82 ± 2.52 mmol/L (n = 16) after 4 weeks of 1% cholesterol feeding. After switching to 0.5% cholesterol feeding for further 12 weeks, total cholesterol concentrations slightly decreased and then remained on a high level (37,10 ± 3.06 mmol/L). It decreased by about 50% during 12 weeks of treatment with lovastatin, although the cholesterol-enriched diet was continued (46.28 ± 6.20 and 23.38 ± 1.34 mmol/L at 4 and 16 weeks, respectively; $P<0.05$), and was significantly lower than those in cholesterol-16 group.

The changes in LDL-cholesterol concentrations went parallel to the plasma total cholesterol levels in all groups. There were no significant differences in HDL-cholesterol concentrations at any time point between any of the groups.

Cu^{2+}-catalyzed oxidation of LDL ex vivo

Figure 1 shows the typical curves of in vitro LDL oxidation after the addition of $CuSO_4$ monitored as the formation of conjugated dienes of LDL. This figure clearly shows the effect of cholesterol-enriched diet on an increased susceptibility of LDL to oxidation, as demonstrated by short lag time, elevated total and rate of diene production.

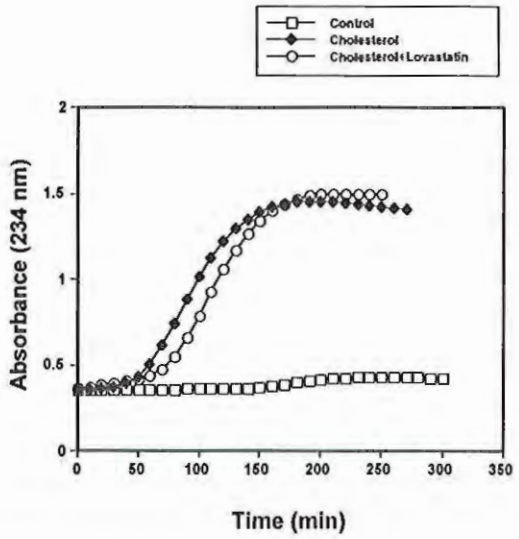

Figure.1 The example of the kinetic absorbance curves of LDL isolated from the one rabbits of the control-16, cholesterol-16, or lovastatin groups at 16 weeks. Effects of the cholesterol feeding on the maximal and rates of diene production are clearly shown.

The lag time of LDL oxidation obtained from rabbits fed a 1% cholesterol-containing diet for 4 weeks was significantly shorter as compared to that observed in the rabbits fed normal chow (85.8 ± 5.3 vs. 161.8 ± 5.0 min; $P<0.05$; Figure 2). After additional 12 weeks of a 0.5% cholesterol-enriched diet, the lag time was 118.9 ± 11.3 min, as compared to 183.0 ± 4.0 min in the control-16 group. Treatment with lovastatin did not result in a significantly different lag time

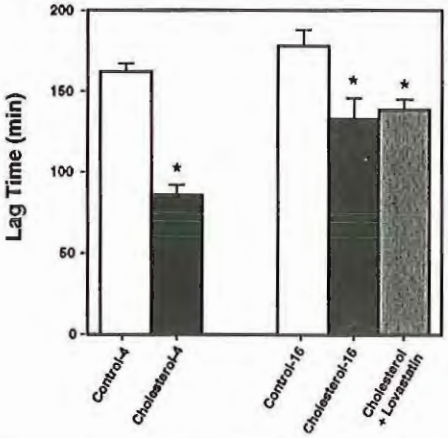

Figure 2 Susceptibility of rabbit-LDL to copper mediated oxidation before (at 4 weeks) and after (at 16 weeks) lovastatin treatment. The resistance of LDL to oxidation decreased markely after 4 or 16 weeks of cholesterol-enriched diet. No significant difference between the cholesterol-16 and lovastatin groups are observed. Data are mean ± S.E.M. * P<0.05 vs. Control

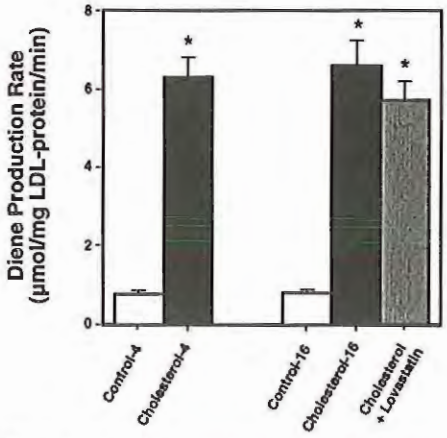

Figure 3 The maximal oxidation rate of LDL derived from rabbits in each groups, exposed to copper. The values were calculated from the slope of kinetic absorbance curve. After 4 or 16 weeks of cholesterol-enriched diet, the rates of diene production increased significantly, as compared to the control group. Lovastatin treatment did not induce significant difference from the cholesterol-16 group. Data are mean ± S.E.M. * P<0.05 vs. Control

as compared to rabbits fed the cholesterol-enriched diet alone (138.3 ± 5.4 min vs. 118.9 ± 11.3 min; P = n.s.).

Concomitantly, the maximal rate of diene production was significantly increased in cholesterol-fed rabbits (Figure 3). In the control group, the mean oxidation rates were 0.75 ± 0.08 and 0.77 ± 0.12 µmol/min/mg LDL protein for the time of 4 and 16 weeks, respectively. The cholesterol diet was associated with a nine-fold elevation in the maximal rate of diene production (6.28 ± 0.52 and 6.59 ± 0.66 µmol/min/mg LDL-protein in the cholesterol-4 and 16 groups, respectively; each P<0.05 vs control). Administration of lovastatin in the last 12 weeks did not significantly change the high rate of diene production as compared to the cholesterol-4 and -16 groups (5.72 ± 0.47 µmol/min/mg LDL protein).

Total diene production was 208.81 ± 6.26 and 261.25 ± 18.74 µmol/mg LDL-protein in the control-4 and -16 groups (Figure 4). Cholesterol

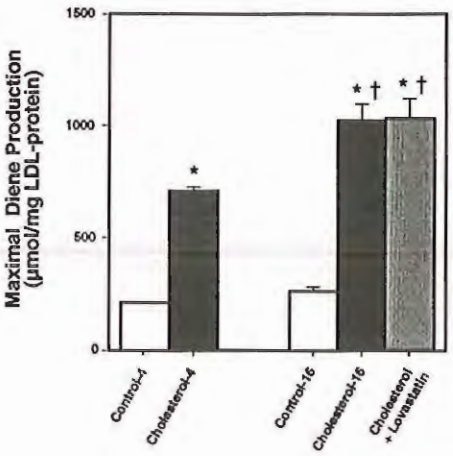

Figure 4 The maximal production of conjugated dienes during the LDL oxidation induced by copper ex vivo. The concentration of conjugated dienes was calculated using the extinction coefficient of 2.8 $x10^4$ $M^{-1}cm^{-1}$. The maximal diend production significantly increased after 4 weeks of a 1% cholesterol-enriched diet and progressively increased after the further 12 weeks of 0.5% cholesterol-enriched diet. There is no significant difference in conjugated diene production between the cholesterol-16 and lovastatin groups. Data are mean ± S.E.M. * P < 0.05 vs. Control, † P<0.05 vs. cholesterol-4 group

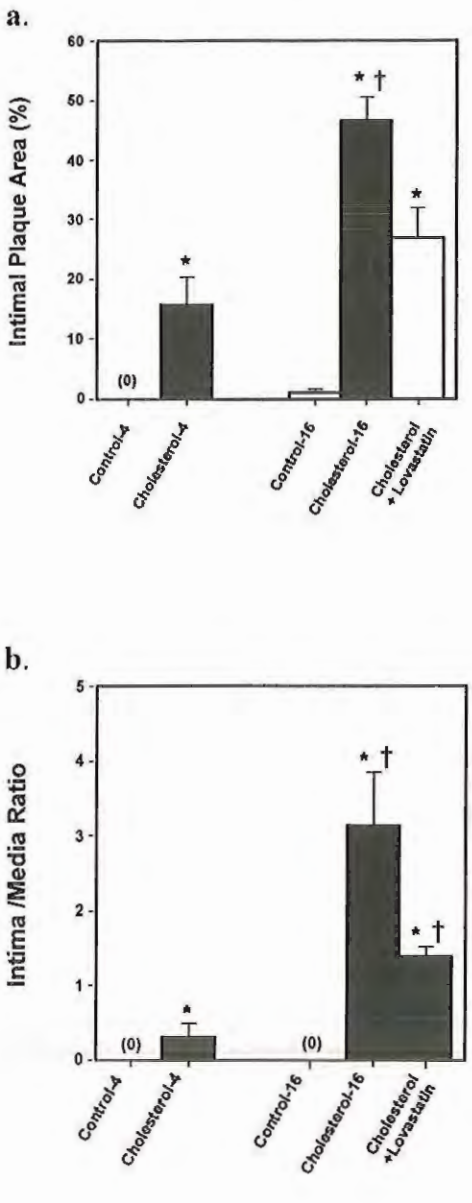

Figure 5 The effect of lovastatin treatment on the carotid arterial intimal plaque area (a) and aortic intimal thickening (b). At the end of treatment period in each groups, the rabbits were sacrified. The intimal lesion of the carotid arteries were assessed by planimetry of digitized photographys and aortic intima/media ratios were assessed by micromorphometry of semithin sections of the thoracic aortas stained with hematoxylineosin. The plague formation increased significantly in the groups of rabbits fed cholesterol-enriched diet. Treatment with lovastatin can slow the progression of the plaque formation and shows significantly difference from those in the cholesterol-16 group. Data are mean ± S.E.M. * P<0.05 vs. Control † P<0.05 vs. cholesterol-4 group

feeding for 4 weeks induced a three-fold increase in the production of diene (703.51 ± 22.3 μmol/mg LDL-protein), which was significantly different from the control-4 group (P<0.05). After further 12 weeks of 0.5% cholesterol feeding, the diene production increased further to 1021.14 ± 91.48 μmol/mg LDL-protein (P<0.05 vs control-4 and cholesterol-4 groups). Treatment with lovastatin had no effect on the total production of diene (1032.78 ± 91.48 μ mol/mg LDL-protein) as compared to the cholesterol-16 group (P = n.s.).

Morphometric and histological analyses

The percentage of intimal plaques in the carotid arteries and aortic intima/media ratios are shown in Figure 5a and 5b. No plaques were found in common carotid arteries in the control-4 group. After 4 weeks of cholesterol feeding, plaques covered 15.8 ± 4.6 % of the total intimal area. Plaque formation progressed to 46.6 ± 4.1 % after additional 12 weeks of 0.5% cholesterol feeding (cholesterol-16 group), but remained low in the control-16 group (0.9 ± 0.6%; P<0.05). Lovastatin treatment nearly abolished the progression of intimal plaque formation (27.0 ± 5.1 %; P<0.05 vs. cholesterol-16 group). It was slightly, but not significantly higher than in the cholesterol-4 group. The percentage of carotid arterial intimal plaque areas were significantly correlated with plasma total cholesterol (r = 0.597, P<0.02; Figure 6) but not with the measures of LDL-oxidation ex vivo.

In both control groups, no intimal thickening of thoracic aortic cross sections was detectable. Cholesterol feeding significantly and progressively increased intima/media ratios to 0.3±0.2 (4 weeks) and 3.1±0.7 (16 weeks). Lovastatin treatment significantly reduced the the intima/media ratios (1.4±0.1; P<0.05 vs. cholesterol 16 group).

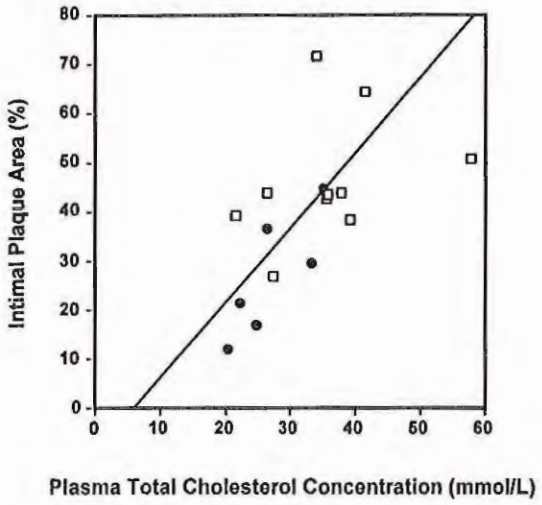

Figure 6 Correlation between plasma total cholesterol concentrations and the carotid arterial intimal plaque area in cholesterol-fed rabbits for 16 weeks treated (●) or untreated (□) with lovastatin. Each value represents a single animals. a) $r = 0.597$, $P<0.02$

DISCUSSION

We have demonstrated that lovastatin treatment suppresses the progression of plaque formation in the carotid arteries in rabbits fed a high cholesterol-diet, while it did not improve oxidative susceptibility of LDL. In the present study, lovastatin was administered for 12 weeks to rabbits after they had already been fed a 1% cholesterol diet for 4 weeks.

Due to inability to compensate for increased input of cholesterol by raising the excretion of cholesterol into the bile, the rabbit is well known among experimental animal species for its tendency to develop severe hypercholesterolemia when dietary cholesterol is increased[29], and to produce a strong relationship between the levels of plasma cholesterol and the degree of aortic lesions with a marked thickening of the intima[2]. In the present study, plasma total cholesterol concentration markedly increased by 41-fold in rabbits receiving 1% cholesterol for 4 weeks, and was maintained on this high level throughout the experimental period, even though the cholesterol content in diet was reduced to 0.5% during the last 12 weeks. This increase was mainly due to an increase in LDL-cholesterol, whereas no changes in the HDL-cholesterol were observed. Treatment with 10 mg/day of lovastatin in hypercholesterolemic rabbits reduced plasma total cholesterol and LDL-cholesterol by about 50%. These data are consistent with previous studies[22,30].

Hypercholesterolemia is one risk factor of atherosclerosis, in which oxidative modification of LDL plays a crucial role. Oxidatively modified LDL may impair the function of signal transduction pathways in endothelial cells, and stimulate many growth factors[9-11]. Monocyte-derived macrophages take up oxLDL by scavenger receptors more rapidly than native LDL[5-7]. There has been much interest in the modulation of oxidative susceptibility of LDL by antiatherosclerotic agents. It is assumed that the lower the resistance of plasma LDL to oxidation, the greater the chance of developing atherosclerotic disease. While the method to detect in vivo LDL oxidation remains unclear, LDL can be oxidized by traces of transition metal ions in a cell-free system ex vivo, particularly effective are Cu^{2+} ions. LDL extracted from human and rabbit lesions was shown to resemble LDL oxidized in vitro by Cu^{2+}[31,32]. In the current investigation we used the method of ex vivo oxidation of LDL by Cu^{2+} ions, which was proved by O'Leary and co-workers[14] to be a reliable index to study the effects of drugs on LDL oxidation. In the present study, neither lag time, maximal diene production nor diene production rate was changed by lovastatin treatment. This finding is in accordance with the result of Yoshida et al.[26] who found no effect of low dose

simvastatin (5 mg/day) on oxidative susceptibility of LDL in hypercholesterolemic patients. However, antioxidant effects of HMG-CoA reductase inhibitors have been previously reported both in vitro and in vivo[23-25]. Kleinveld et al.[25] showed that simvastatin and pravastatin altered the composition of LDL derived from plasma of hypercholesterolemic patients and resulted in its reduced oxidizability by decreasing total and maximal rate of diene production with unchanged lag time. In contrast, a study by Aviram et al.[23] in hypercholesterolemic patients demonstrated antioxidant properties of lovastatin both in vitro and in vivo without any change in LDL composition, and suggested that lovastatin might bind to the LDL surface and alter polyunsaturated fatty acid availability to in vitro oxidation. This discussion is still controversial. In addition, these studies were performed in patients with mild hypercholesterolemia without atherosclerosis. There is no previous report on antioxidant properties of lovastatin in animals. In the present experiment, we studied the effects of lovastatin in rabbits with severe hypercholesterolemia (total cholesterol concentration of 46.82 ± 2.52 mmol/L) and pre-established atherosclerotic lesions (carotid intimal plaque area of 15.8 ± 4.6% and aortic intima/media ratio of 0.3±0.2). Moreover, various compounds within the group of HMG-CoA reductase inhibitors have been shown to possess varying degrees of hepatoselectivity and plasma cholesterol lowering[30]. Therefore, a compound specific effect may be one cause for the different results.

A study of Gilligan et al.[33] showed that there is a strong negative correlation of the lag time of LDL oxidation with the levels of plasma cholesterol in hypercholesterolemic patients. A similar result was found in the prsent study. Lipoproteins derived from hypercholesterolemic patients contain increased concentrations of cholesterol, resulting in enhanced in vitro susceptibility of LDL for oxidation[13,34]. The lack of effect of lovastatin on LDL oxidation in our present study suggests that other components of the LDL particle beyond cholesterol influence the susceptibility of LDL to oxidation, which are not influenced by lovastatin treatment. There are evidences that oleate-enriched diets reduce the susceptibility of LDL to oxidation, whereas linoleate-enriched diets increase the susceptibility[35]. In addition, smaller, more dense LDLs display enhanced susceptibility to Cu^{2+}-induced oxidation when compared to larger, more buoyant lipoprotein particles[36].

The earliest histologically recognizable atherosclerotic lesion is the fatty streak. It is clearly shown that a decrease of plasma lipid levels can reduce atherosclerotic lesion formation[2,20,21]. In the current investigation, after 4 weeks of cholesterol feeding, intimal plaque in the carotid arteries and intimal thickening in aorta significantly increased and aggressively progressed when the cholesterol-feeding was further continued for 12 weeks. Dietary supplementation with lovastatin decreased these progression despite continued intake of the high-cholesterol diet. Intimal plaque formation significantly correlated with plasma total cholesterol concentrations. The beneficial effect seen with lovastatin therefore seemed to be due to its cholesterol-lowering effect. A reduction in lesion size of atherosclerotic arteries in high cholesterol-fed cynomolgus monkeys was seen after the cholesterol level was decreased by returning to a normal diet[37]. Extent and type of atherosclerotic lesions induced in rabbits has been shown to be dependent upon the overall plasma cholesterol exposure, VLDL- and LDL-cholesterol content[2]. Soma et al.[38]

reported that lovastatin administered at 20 mg/kg inhibited neointimal formation by 33% in normocholesterolemic rabbits when lesions were induced by surrounding the carotid artery with a flexible collar. In other studies[20] in which lovastatin therapy was initiated simultaneously with the cholesterol diet and plasma cholesterol levels were maintained relatively low, lovastatin was found to prevent atherosclerotic lesion formation. Similar results have been reported by Bocan et al.[2] who found a development of minimal atherosclerosis in the thoracic aorta of rabbits when cholesterol levels were maintained below 700 mg/dL. A regression of aortic atherosclerosis in cholesterol-fed rabbits was found by the study of Zhu et al.[39] who used a high dose of lovastatin (6 mg/kg/day) combined with a normal diet, inducing a reduction of plasma cholesterol levels by 95%.

In addition, there is evidence that mevalonate and other intermediates (isoprenoids) of cholesterol synthesis are essential for DNA synthesis and cell proliferation[40]. Thus, inhibition of HMG-CoA reductase may prevent an increase in DNA synthesis induced by platelet-derived growth factor, and subsequent cell growth. It is therefore possible that the beneficial antiatherosclerotic effect of lovastatin is due in part to reduced intimal hyperplasia by inhibiting smooth muscle cell proliferation. Previous observations demonstrated that treatment of cells with lovastatin and compactin resulted in growth arrest unrelated to the presence of cholesterol[41]. Subsequently, lovastatin, fluvastatin, and simvastatin have been shown to dose-dependently decrease smooth muscle cell migration and proliferation in cell culture[42]. On the other hand, one study showed that administration of lovastatin to hypercholesterolemic rabbits markedly attenuated both the reduced basal NO production and the increased adhesiveness of the endothelium[43]. More recently, lovastatin has been shown to reduce the expression of scavenger receptor CD36 in human monocytic cells[44].

In conclusion, the results show that lovastatin can slow the progression of intimal plaque lesions in the carotid arteries even in the presence of preestablished lesions. This effect is not associated with a decreased susceptibility of LDL to Cu^{2+}-mediated LDL oxidation ex vivo.

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