Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1185–1191
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Effect of simvastatin on the oxidation of native and modified lipoproteins

Grazyna Sobal *, Helmut Sinzinger

Department of Nuclear Medicine, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria

Received 25 May 2005; accepted 18 July 2005

Abstract

Modified (oxidized) low-density lipoprotein (LDL) plays a significant role in atherosclerosis by accumulation in arteries. Also, glycated LDL, such as in diabetics, are increasing the risk for atherosclerosis, due to an increased oxidizability as compared to native LDL. For these reasons, the potential inhibition of such modifications is of clinical importance. We investigated the influence of simvastatin on oxidation of native and modified LDL as well as high-density lipoprotein (HDL), which plays a protective role in atherosclerosis. Quantitative assessment of the oxidation end-product malondialdehyde (MDA) revealed the highest inhibitory rate for HDL at concentrations of 1.6 μg/ml and 0.8 μg/ml by 30.3% and 20.4%, at 6 h and 4 h, respectively. At 24 h, the inhibition was still persisting amounting to 27.9% and 20.3%, respectively.

For native LDL, we found less inhibition of oxidation at a concentration of 1.6 μg/ml amounting to 19.2% and 11.5%, for 4 h and 6 h, respectively. Similar effects were found at a concentration of 0.8 μg/ml. For modified, glycated LDL, the most pronounced effect was found at a concentration of 1.6 μg/ml amounting to 22.4% for the period of 2–24 h of oxidation. For glycoxidated LDL, the inhibition of oxidation was less expressed amounting to 10.1% for the period of 2–6 h at the same concentration.

The influence of simvastatin on lag time (protection from oxidation) by diene conjugation was also investigated. At the highest concentration of simvastatin (1.6 μg/ml), we found a prolongation of lag time from 73 min to 99 min for native LDL, for glycoxidated LDL 60 min to 89 min and for HDL 54 min to 64 min. For glycated LDL, only a small decrease of lag time (66 min versus 71 min) at same concentration was observed. For glycated and glycoxidated LDL, we found a moderate increase in relative electrophoretic mobility (REM) by 2.0 and 2.3, respectively, but no changes in the presence of simvastatin were observed. These data show that simvastatin besides its lipid-lowering action has also significant antioxidative properties.

Keywords: Lipoproteins; Modified lipoproteins; Glycation; Glycoxidation; Statins; Simvastatin; Atherosclerosis

1. Introduction

Primary hypercholesterolemia is a key risk factor for development of atherosclerosis and coronary heart disease (CHD). Low-density lipoproteins (LDL), especially in modified forms (oxidized or glycated = non-enzymatically glycosylated), are favouring atherosclerosis, due to enhanced accumulation in the arterial wall and formation of ''foam cells'' [1–3]. The non-enzymatic glycosylation of LDL is accelerated in patients with diabetes mellitus. There is also evidence that glycated LDL is more prone to oxidation than native LDL [4–6]. Since LDL-oxidation and/or -glycation seems to play such a prominent role in the pathophysiology of atherosclerosis, it is evident that lipid-lowering agents, such as statins, could be of benefit in the treatment of the disease. Simvastatin is a member of the statins family of such as lovastatin, pravastatin, fluvastatin, atorvastatin and rosuvastatin in the treatment of hypercholesterolemia [7–10]. These drugs are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme, which is the rate limiting step in the biosynthesis pathway of cholesterol. Simvastatin, the most widely used compound, effectively reduced total plasma cholesterol and LDL-cholesterol levels by 25–35% [11–14]. Reduction of triglyceride

Abbreviations: Apo I, apolipoprotein I; BHT, butylated hydroxytoluene; CHD, coronary heart disease; CRP, C-reactive protein; EDTA, ethylenediaminetetraacetic acid; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; MDA, malondialdehyde; PBS, phosphate-buffered saline; REM, relative electrophoretic mobility; TBA, thiobarbituric acid; TCA, trichloroacetic acid; VLDL, very low-density lipoprotein

* Corresponding author. Tel.: +43 1 40400 5558; fax: +43 1 40400 5552.
E-mail address: Grazyna.Sobal@meduniwien.ac.at (G. Sobal).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.07.022

was less pronounced by 7–30%, mildly raises high-density lipoprotein (HDL)-cholesterol was increased by 5–15% [15]. The changes in HDL-cholesterol were even more pronounced by 29.1% versus 11.4% in patients with low versus normal HDL under a high-dose of 80 mg simvastatin treatment [16]. HDL plays an increasingly important role as a protective factor in atherogenesis, due to the reverse cholesterol transport from peripheral cells to the liver. Representative data indicate that a large number of patients with CHD even with normal LDL cholesterol ($\leq$100–130 mg/dl) show a subnormal level of HDL-cholesterol [17]. In older people, HDL is more predictive for a vascular event as compared to LDL-cholesterol. The Framingham study [18] shows that for each 1 mg/dl increase in HDL, a 2–3% decrease in CHD occurred. In this study, low HDL cholesterol is associated with high level of atherogenic lipoproteins such as very low-density lipoprotein (VLDL), small dense LDL, metabolic syndrome and hypertension [19]. The studies show that statins are able to increase HDL and apolipoprotein I (Apo I) levels for both healthy and CHD individuals [20–25]. This is a very important fact, because both parameters are responsible for removal and transfer of cholesterol from the artery wall to the liver. Statins besides the lipid-lowering effects have also been demonstrated to have a variety of non-lipid effects being of key relevance in atherogenesis. Among them are a decreased smooth muscle cell proliferation and endothelial deactivation, reduction in C-reactive protein (CRP) and also antithrombotic effects [26]. Isoprostane measurement in patients with hyperlipidemia demonstrated an antioxidative action of statins [27].

Data on the antioxidative effect of statins on LDL and even more on HDL oxidation are rare. For this reason, we investigated the effect of simvastatin on oxidation of native and modified LDL and also on oxidation of HDL.

2. Materials and methods

2.1. Simvastatin

Simvastatin was kindly provided by Lannacher Heilmittel Gmbh (Lannach, Austria). The drug was used as a stock solution in ethanol (16 mg/ml).

2.2. Isolation of LDL and HDL

Blood from healthy normolipidemic overnight fasting volunteers ($n = 8$, non-smokers, non-diabetics, not taking any drug for at least 2 weeks) aged 25–55 years was collected into syringes containing sodium ethylenediaminetetraacetic acid (EDTA) (4 mM). Native LDL and HDL were isolated by isopycnic preparative ultracentrifugation (Sorvall ETD, SW-41 swing out rotor, 41,000 rpm, 10 °C, 24 h) of plasma using a discontinuous KBr density gradient according to Chapman et al. [28].

2.3. Preparation and characterisation of LDL modifications

For oxidation or glycation experiments, stock solutions were dialysed extensively in the cold against phosphate-buffered saline (PBS), pH 7.4. For glycation, dialysed LDL at a concentration of 0.6–0.7 mg LDL protein/ml was incubated with 0.5 M glucose in the presence or absence of antioxidants (1.0 mM EDTA, 20 μM butylated hydroxytoluene (BHT)) at 37 °C for 4 weeks in the dark (''g'' versus ''go'' LDL). Advanced glycosylation end products (AGEs) formation was monitored by measuring the fluorescence at 370 nm excitation/440 nm emission on a Hitachi RF-551 S spectrofluorometer. Only fluorescence spectra showing the characteristic maximal emission at 440 nm were considered as AGE-specific. Oxidative modification of LDL also results in the generation of fluorescence peaking at 430 nm (excitation at 360 nm), so that glycoxidative changes are manifested by combined oxidative/AGE-specific fluorescence. Fluorescence data are given in relative fluorescence units (RFU) of 0.1% LDL solutions. After 4 weeks of glycation/glycoxidation, mean AGE–RFU values for gLDL and goLDL were 310 RFU and 540 RFU, respectively.

2.4. Measurement of oxidation parameters of LDL

2.4.1. Formation of conjugated dienes (CD) and baseline dienes (BD)

Isolated LDL and HDL were chromatographed over a PD-10 size exclusion column (Pharmacia Biotech Sephadex G-25M gel filtration column) preequilibrated in PBS to remove EDTA and BHT and then diluted to a final concentration of 0.25 mg/ml mass and absorbance read at 234 nm (Hitachi U-2000 spectrophotometer, Osaka, Japan) to calculate BD, using a molar absorption coefficient of 29,500 M^{-1} cm^{-1}. Oxidation was initiated by addition of an aqueous copper-sulfate solution to a final concentration of 5 μmol/l. Immediately after addition of copper, absorbance at 234 nm was followed at room temperature over 300 min in 5 min intervals to obtain a typical CD-formation curve as described by Esterbauer et al. [29,30]. The lag time, defined as end of the crosspoint of the time axis and the curve slope, was assessed.

2.4.2. Thiobarbituric reactive substances (TBARS)

Lipid peroxidation was determined by quantification of TBARS with a microtiter plate-modification of the method described by Buege and Aust [31]. Briefly, 50 μl 50% (w/v) (trichloroacetic acid (TCA), Merck), 75 μl of 1.3% (w/v) (thiobarbituric acid (TBA), Merck) dissolved in 0.3% NaOH (Merck), 60 μl of PBS and 100 μl of LDL sample were mixed in a 96-well microtiter plate (Sterilin), covered

and placed in a water-bath for 40 min at 60 °C. After cooling on ice, plates were centrifuged at 2000 rpm for 10 min in a Hettich Rotixa RP centrifuge (Hettich, Darmstadt, Germany) and 200 μl of the supernatant of each well pipetted into corresponding wells in a second plate. Absorbance at 530 nm was determined in an Anthos HT II microtiter plate reader (Anthos Labtec, Salzburg, Austria). TBARS concentration was calculated using a malonyldialdehyde (MDA) standard curve and expressed as nanomolar MDA-equivalent per milligram LDL protein.

2.4.3. Relative electrophoretic mobility (REM)

Gel-electrophoresis was performed on agarose gels provided by Baxter AG (Vienna, Austria). 0.25 mg/ml of LDL samples were applied to the gel. Electrophoresis was performed at 300 mA for 120 min. Protein bands were stained with amido black and REM was calculated as the quotient of running distance of modified and native LDL.

2.5. Statistical analysis

The results are expressed as mean values ± S.D. Statistical analysis was performed using one-way ANOVA. A $p < 0.05$ was considered statistically significant.

3. Results

We found dose-dependent influence of simvastatin on oxidation of native and modified LDL and HDL as well. Interestingly, we observed an inhibitory action on oxidation of different forms of LDL and HDL for a period up to 24 h. The highest inhibitiory rate of oxidation was found for HDL, at concentrations of 0.1 μg/ml, 0.8 μg/ml and 1.6 μg/ml for the whole period of 2–24 h (Fig. 1). Quantification of the oxidation end-product malondialdehyde (MDA) revealed the maximum of inhibition at concentrations of 1.6 μg/ml and 0.8 μg/ml by 30.3% and 20.4%, at 6 h and 4 h, respectively. At 24 h, the inhibition was still persisting (27.9% and 20.3%, respectively). For native LDL, we found a comparable maximum of oxidation inhibition for 4 h and 6 h, at a concentration of 1.6 μg/ml, amounting to 19.2% and 11.5%, respectively. Similar effects were found at a concentration of 0.8 μg/ml (Fig. 2).

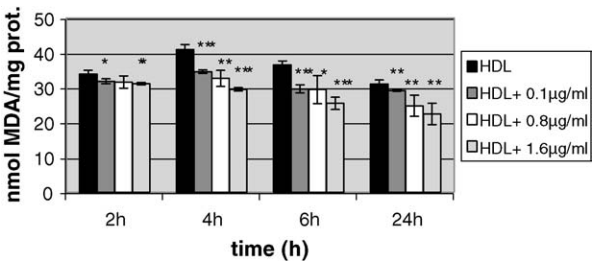

Fig. 1. Copper-mediated oxidation of HDL in the presence of different concentrations of simvastatin, as monitored by oxidation end-product malondialdehyde (MDA). Data are given as mean values ± S.D. of three independent experiments with $^{*}p < 0.01$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ vs. HDL in absence of simvastatin.

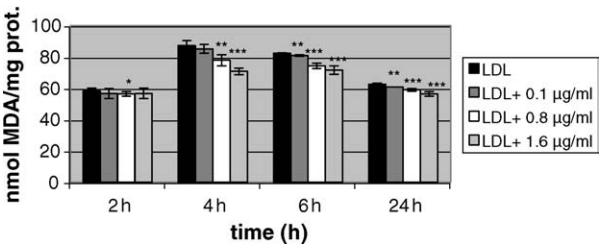

Fig. 2. Copper-mediated oxidation of nLDL in the presence of different concentrations of simvastatin, as monitored by oxidation end-product malondialdehyde (MDA). Data are given as mean values ± S.D. of three independent experiments with $^{*}p < 0.01$, $^{**}p < 0.001$ and $^{***}p < 0.0001$ vs. nLDL in absence of simvastatin.

For modified, glycated and glycoxidated LDL, which plays an important role in diabetics, we found also an inhibition of oxidation. The most pronounced effect was found for glycated LDL during the whole period of oxidation of 2–24 h (Fig. 3). For glycated LDL, the inhibition of oxidation was expressed to a maximum of 14.9% at a concentration of 0.8 μg/ml and 13.6% at a concentration of 0.1 μg/ml at 6 h. At a concentration of 1.6 μg/ml, the maximum of inhibition was achieved with 22.4% for the period of 2–24 h of oxidation. For glycoxidated LDL, the inhibition of oxidation was less expressed amounting to 3.7–10.1% for the period of 2–6 h at a concentration of 1.6 μg/ml and about 6% at concentration of 0.1 μg/ml for the same period (Fig. 4).

We investigated not only the influence of simvastatin on generation of oxidation end-product MDA, but also on lag time (protection from oxidation) before the initiation of oxidation took place. For native LDL, we found a prolongation of lag time from 73 min to 99 min in the presence of simvastatin at the highest concentration of 1.6 μg/ml (Fig. 5). At the lowest concentration of 0.1 μg/ml, we found only an insignificant prolongation by 5% of the mean. For glycated LDL, we have even observed some decrease of lag time (66 min versus 71 min) at the highest concentration of 1.6 μg/ml (Fig. 6). At the lowest concentration of 0.1 μg/ml, the lag time was unchanged.

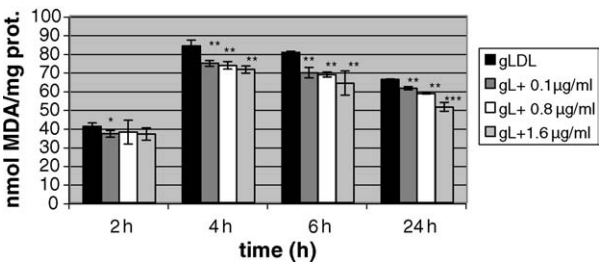

Fig. 3. Copper-mediated oxidation of gLDL in the presence of different concentrations of simvastatin, as monitored by oxidation end-product malondialdehyde (MDA). Data are given as mean values ± S.D. of three independent experiments with $^{*}p < 0.01$ and $^{**}p < 0.001$ vs. gLDL in absence of simvastatin.

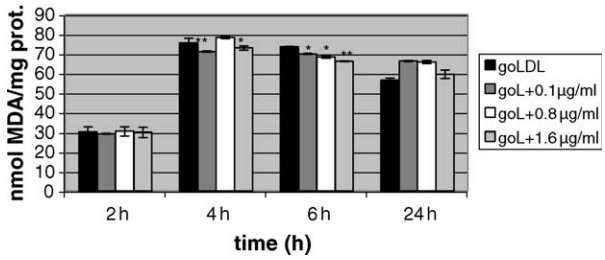

Fig. 4. Copper-mediated oxidation of goLDL in the presence of different concentrations of simvastatin, as monitored by oxidation end-product malondialdehyde (MDA). Data are given as mean values ± S.D. of three independent experiments with $^{*}p < 0.01$ and $^{**}p < 0.001$ vs. goLDL in absence of simvastatin.

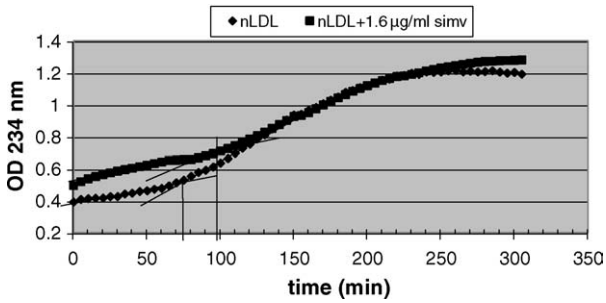

Fig. 5. Influence of simvastatin on lag time for native LDL as maximal effect at the highest concentration of 1.6 μg/ml. Native LDL was diluted to standard concentration (0.25 mg/ml total cholesterol) and oxidation initiated by addition copper-sulfate solution with a final concentration of 5 μmol/l. Absorbance at 234 nm was followed at room temperature over 300 min in 5 min intervals in a Hitachi U-2000 spectrophotometer to obtain a typical conjugated diene-formation (CD) curve. From CD-formation curve, the lag time defined as end of the crosspoint of the time axis and the curve slope was estimated.

For glycoxidated LDL, we observed a prolongation of lag time (89 min versus 60 min) at the highest concentration of 1.6 μg/ml (Fig. 7) and no change at the lower concentrations. For HDL, we found beneficial influence on the lag time at the highest concentration of 1.6 μg/ml (73 min versus 54 min) (Fig. 8) and no changes at the lower concentrations.

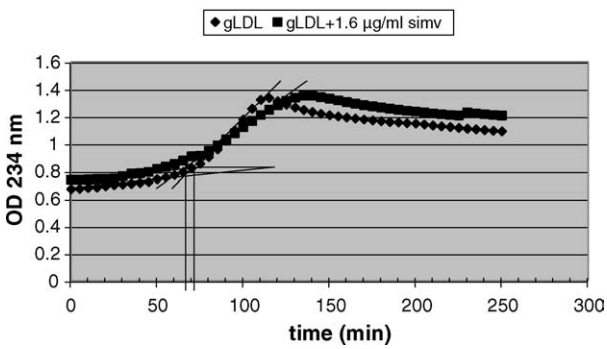

Fig. 6. Influence of simvastatin on lag time for gLDL as maximal effect at the highest concentration of 1.6 μg/ml. The oxidation for gLDL and conjugated diene-formation was estimated at the same condition as for nLDL.

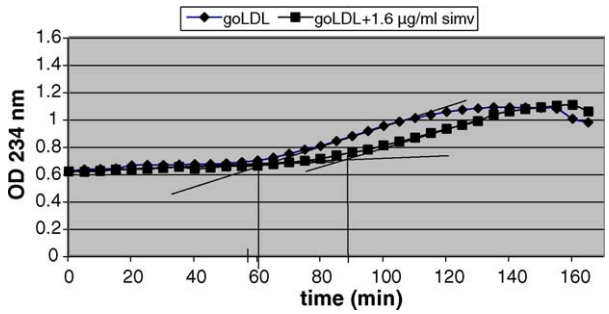

Fig. 7. Influence of simvastatin on lag time for goLDL as maximal effect at the highest concentration of 1.6 μg/ml. The oxidation for gLDL and conjugated diene-formation was estimated at the same condition as for nLDL.

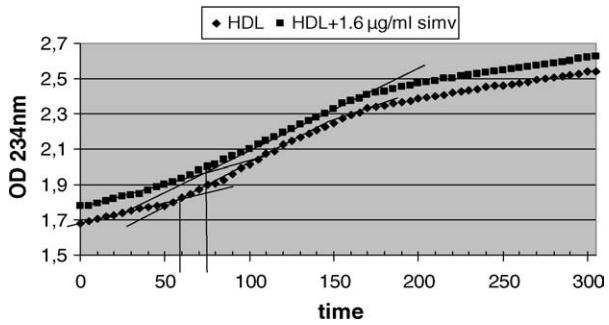

Fig. 8. Influence of simvastatin on lag time for HDL as maximal effect at the highest concentration of 1.6 μg/ml. The oxidation for gLDL and conjugated diene-formation was estimated at the same condition as for nLDL.

The influence of simvastatin on 4 h oxidation of native and modified LDL was investigated by agarose electrophoresis. For glycated and glycoxidated LDL, we found a moderate increase in REM by 2.0 and 2.3, respectively, but no changes in presence of simvastatin were observed.

4. Discussion

The most pronounced inhibition of oxidation in presence of simvastatin was found for HDL. This lipoprotein plays an important protective role in atherosclerosis, acting against cholesterol accumulation in the foam cells, by promoting of its efflux.

Oxidation of HDL is at least as important as that of LDL in respect to atherogenesis. The Apo A-I of HDL is also sensitive to oxidation like Apo B in LDL. This process leads to molecular modifications and changes in its metabolism (scavenger receptors). This results in a decreased cholesterol efflux from peripheral tissues [32,33] and could thus contribute to atherogenesis.

The protection of HDL oxidation will maintain the molecule in a native, not modified state promoting its original role as a protecting factor. This is of key importance, as the studies with human macrophages demonstrated that oxidized HDL (for 12 h and 24 h) decreases the

efflux of intracellular free cholesterol by 16–45% [34]. This means that HDL loses its atheroprotective action as a consequence of oxidation. According to our findings, the inhibition of oxidation was persistent for a very long time of up to 24 h. Also, native LDL was protected from oxidation up to 19.2% for a quite long period of up to 6–24 h. Glycated LDL was protected to a comparable extent as native LDL amounting to 14.9%. We found less protection against oxidation for glycoxidated LDL. This is a well-known fact that this form of LDL is oxidatively more modified and exposed to higher oxidative stress than native LDL.

Our results show that the antioxidative action of simvastatin is addressed to long protection of oxidation of various LDL forms and HDL. The bioavailability of simvastatin has been reported to be low [35–37,14]. About 5% of the dose of simvastatin is available in circulation as active drug or metabolites in humans. The inhibition was achieved for all forms of LDL and HDL at concentrations corresponding to plasma levels of simvastatin achieved in the therapeutic range of 10 mg up to 160 mg daily considering a bioavailability of 5%. The plasma level in the treatment phase at these dosages corresponds to the experimental concentrations of 0.1–1.6 μg/ml. This very accurate calculation is very important, because it is well established that simvastatin especially at higher concentrations causes side effects like myopathy [38].

Our experimental data show that simvastatin besides its lipid-lowering action has also significant antioxidative properties. The results provide evidence that the antioxidative effects of simvastatin in the concentration range 0.1–1.6 μg/ml are most pronounced during the longer time period of action 6–24 h. It seems that this drug exerts some of its action by antioxidative properties. By lag time estimations (diene conjugation), the protective effects were seen starting slowly with the initiation of oxidation of native or glycated LDL in the first 1–2 h in the investigated concentration range of 0.1–1.6 μg/ml. In case of glycated LDL, only a weak protective effect on lag time was found. These results provide evidence that simvastatin probably because of its chemical structure is able to protect the native or modified LDL from the generation of lipid radicals and conjugated diene in the initial phase of oxidation. The protective antioxidative effect was also found in the later phase of lipids peroxidation sequelae, when the lipid peroxidation end products were generated, e.g. MDA. The reduction of MDA we have seen quite expressed by native and glycated LDL, but a special strong in case of HDL.

The structure of simvastatin [35] consists of two conjugated phenolic rings, which are known as a free radicals scavenger source, especially for very active $OH^{\bullet}$ radicals. It also has a conjugated diene structure by two double bonds by adjacent carbon atoms, which is very prone to formation of peroxides. Furthermore, there are also two oxygen double bonds (on the phenolic ring and aliphatic chain), which could be a source for O_2^- radicals by a possible formation of peroxides or hydroxyperoxides. This antioxidative mechanism is also supported by the metabolic pathway of simvastatin. In vivo simvastatin is converted to bioactive compounds by oxidation steps including formation of aldehyde and finally acidic compound 6′β-carboxy simvastatin. These are our considerations and speculations, how simvastatin could protect lipids from oxidation by a free radicals attack, undergoing oxidative modification itself.

The antioxidant effects of simvastatin on glycated and glycoxidated LDL are very important, as diabetics have increased risk for atherosclerosis [39,40] and glycated LDL is more sensitive to oxidation than native LDL [4–6,41]. After 4 weeks of glycation/glycoxidation, mean AGE–RFU values for gLDL and goLDL were 310 RFU and 540 RFU, respectively. Although the AGE–RFU values for gLDL are lower than for goLDL, MDA formation showed similar profile, but still higher for gLDL than for goLDL. One explanation for these results could be the heterogeneity of the blood donors concerning antioxidant status of LDL as well as its prones for glycation and/or glycoxidation. For glycated and glycoxidated LDL, we found a moderate, similar increase in relative electrophoretic mobility by 2.0 and 2.3, respectively, as compared to native LDL. According to evidence in literature [42] in NIDDM patients, Vitamin A, SH-groups and uric acid were significantly reduced, whereas the level of Vitamin E was significantly increased. Vitamin C was similar as compared to healthy subjects. Other study [43] reports lower contents of α-tocopherol and β-carotene in naturally occurring multiple-modified LDL as compared to native LDL.

For glycated LDL, the inhibition of oxidation was at maximum 14.9% at a concentration of 0.8 μg/ml and 13.6% (0.1 μg/ml), for 6 h. There was an even slightly higher inhibition of oxidation for glycated LDL as compared to native LDL. Simvastatin shows the protective antioxidative properties in a comparable manner for both, native and glycated LDL. Our data are also supported by other findings that simvastatin causes significant reduction in plasma total cholesterol and LDL cholesterol which are similar in magnitude for both diabetics and non-diabetics patients [44,45].

It would be interesting to compare the antioxidative potency of simvastatin with other drugs like acetylsalicylic acid, troglitazone and calcium antagonists on their antioxidative potency in previous studies [46–48]. Acetylsalicylic acid shows, only at ‘‘supra therapeutic’’ concentrations in the range of 0.06–2.0 mg/ml, a significant concentration-dependent inhibition of LDL oxidation both for native and glycated LDL (up to 89.6% and 64.4%, respectively). The borderline concentration of 0.06 mg/ml (reflecting an in vivo dose of about 300 mg/d) amounted still to 10.9% and 10.7%, inhibition of oxidation, respec-

tively. From 0.2 mg/ml upwards, a significantly more marked inhibition for native LDL than for glycated LDL was found. Troglitazone was a potent inhibitor of 8-epi-$PGF_{2\alpha}$ formation at a concentration of 5–20 μg/ml. At a concentration of 5 μg/ml corresponding to the highest therapeutic plasma concentration troglitazone inhibited significantly 8-epi-$PGF_{2\alpha}$ formation of LDL and its modifications by 20% for 24 h. At higher concentrations, we have also found high concentration-dependent inhibition of oxidation for native and glycated LDL (almost to the same extent) and for glycoxidated LDL only by half of that for native or glycated LDL (measured by TBARS formation). Calcium antagonists exhibited a high antioxidative effect in the order: lacidipine > semotiadil > isradipine >amlodipine > nifedipine > diltiazem at concentrations 10^{-3} M to 10^{-5} M as measured by TBARS formation. At lower concentrations (10^{-6} M to 10^{-7} M) corresponding to lower therapeutic concentration range (10^{-5} M to 10^{-7} M), we found only a 5–10% not significant inhibition of oxidation for native and modified LDL. Only for lacidipine, the most lipid-soluble calcium antagonist, the higher investigated concentrations of 10^{-3} M, which might be achieved in the microenvironment in vivo, are of relevance.

We found that the antioxidative action of simvastain is persisting up to 6–24 h. Also, HDL protection from oxidation seems to be very important, as HDL is acting against accumulation of modified LDL in the artery wall. The protection of HDL oxidation can be speculated as a reason for HDL-level elevation (in its native, unmodified form) during the treatment with simvastatin, as well documented in the literature [20–25]. The kinetics of modified and native LDL might be different [49,50] eventually influencing circulating levels of HDL. These findings imply a decreased accumulation of modified LDL in the arterial wall and higher HDL level exerting a vasoprotective role. Summarizing, simvastatin evolved a beneficial effect by preventing oxidation of both LDL and HDL exerting thereby a double antiatherogenic action.

References

[1] Steiner C. Atherosclerosis, the major complication of diabetes. Adv Exp Med Biol 1985;189:277–97.

[2] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modification that increases its atherogenicity. New Engl J Med 1989;320:915–24.

[3] Sparrow CP, Parthasarathy S, Steinberg D. Macrophage receptor that recognizes oxidized low-density lipoprotein but not acetylated low-density lipoprotein. J Biol Chem 1989;264:2599–604.

[4] Sobal G, Menzel EJ, Sinzinger H. Why is glycated LDL more sensitive to oxidation than native LDL?—a comparative study. Prostagl Leuk Essent Fatt Acids 2000;63:177–86.

[5] Menzel EJ, Sobal G, Staudinger A. The role of oxidative stress in the long-term glycation of LDL. BioFactors 1997;6:111–24.

[6] Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H. Lipid advanced glycosylation: pathway for lipid oxidation in-vivo. Proc Natl Acad Sci USA 1993;90:6434–8.

[7] Farnier M, Davignon J. Current and future treatment of hyperlipidemia: the role of statins. Am J Cardiol 1998;82:3J–10J.

[8] Hsu I, Spinler SA, Johnson NE. Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. Ann Pharmacother 1995;29:743–59.

[9] Lennernäs H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. Clin Pharmacokinet 1997;32:403–25.

[10] Plosker GL, McTavish D. Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. Drugs 1995;50:334–63.

[11] Desager JP, Horsmans Y. Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. Clin Pharmacokinet 1996;31:348–71.

[12] Mauro VF, MacDonald JL. Simvastatin: a review of its pharmacology and clinical use. DICP 1991;25:257–64.

[13] Pedersen TR, Tobert JA. Benefits and risks of HMG-CoA reductase inhibitors in the prevention of coronary heart disease: a reappraisal. Drug Saf 1996;14:11–24.

[14] Todd PA, Goa KL. Simvastatin. A review of its pharmacological properties and therapeutic potential in hypercholesterolaemia. Drugs 1990;40:583–607.

[15] Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). J Am Med Assoc 2001;285:2486–97.

[16] de-Sauvage-Nolting PR, Buirma RJ, Hutten BA, Kastelein JJ. Baseline lipid values partly determine the response to high-dose simvastatin in patients with familial hypercholesterolemia. The examination of probands and relatives in statin studies with familial hypercholesterolemia. Atherosclerosis 2002;164:347–54.

[17] Amarenco P. Hypercholesterolemia, lipid-lowering agents, and the risk for brain infarction. Neurology 2001;57(Suppl. 2):S35–44.

[18] Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: the Framingham Study. Am J Med 1977;62:707–14.

[19] Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RMJ. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. Clin Invest 1993;92:141–6.

[20] Von Eckardstein A, Nofer J, Assmann G. High density lipoproteins and arteriosclerosis: role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2001;21:13–7.

[21] Kwiterovich Jr PO. The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. Am J Cardiol 2000;86(Suppl.):5L–10L.

[22] Segrest JP, Li L, Anantharamaiah GM, Harvey SC, Liadak KN, Zannis V. Structure and function of apolipoprotein A-I and high density lipoprotein. Curr Opin Lipidol 2000;11:106–15.

[23] Harper CR, Jacobson TA. New perspectives on the management of low levels of high-density lipoprotein cholesterol. Arch Intern Med 1999;159:1049–57.

[24] Kashyap ML. Mechanism studies of high-density lipoproteins. Am J Cardiol 1998;82:42U–8U.

[25] Alan Tall R. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. J Clin Invest 1990;86:379–84.

[26] Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: Implications for cardiovascular event reduction. J Am Med Assoc 1998;279:1643–50.

[27] Sinzinger H, Oguogho A. Variable influence of statins on isoprostanes in hyperlipidemia. Adv Exp Med Biol 2003;525:209–12.

[28] Chapman MJ, Goldstein S, Lagrange D, Laplaud PM. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. J Lipid Res 1981;22:339–58.

[29] Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 1989;6:67–75.
[30] Ahotupa M, Marniemi J, Lehtimäke T, Talvinen K, Raitakari OT, Vasankari T, et al. Baseline diene conjugation in LDL lipids as a direct measure of in vivo LDL oxidation. Clin Biochem 1998;31:257–61.
[31] Buege JA, Aust SD. Lactoperoxidase-catalyzed lipid peroxidation of microsomal and artificial membranes. Biochim Biophys Acta 1976; 444:192–201.
[32] Salmon S, Maziere C, Auclair M, Theron L, Santus R, Maziere JC. Malondialdehyde modification and copper-induced autooxidation of high-density lipoprotein decrease cholesterol efflux from human cultured fibroblasts. Biochim Biophys Acta 1992;1125:230–5.
[33] Matsunaga T, Nakajima T, Miyazaki T, Koyama I, Hokari S, Inoue I, et al. Glycated high-density lipoprotein regulates reactive oxygen species and reactive nitrogen species in endothelial cells. Metabolism 2003;52:42–9.
[34] La Ville AE, Sola R, Balanya J, Turner PR, Masana L. In vitro oxidised HDL is recognized by the scavenger receptor of macrophages: implications for its protective role in vivo. Atherosclerosis 1994;105:179–89.
[35] Christians U, Jacobsen W, Floren LC. Metabolism and drug interactions of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in transplant patients: are the statins mechanistically similar? Pharmacol Ther 1998;80:1–34.
[36] Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. Clin Pharmacokinet 1993;24:195–202.
[37] Plosker GL, McTavish D. Simvastatin. A reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. Drugs 1995;50:334–63.
[38] Rosenson RS. Current overview of statin-induced myopathy. Am J Med 2004;116:408–16.
[39] Steiner C. Atherosclerosis, the major complication of diabetes. Adv Exp Med Biol 1985;189:277–97.
[40] Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A, et al. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. Proc Natl Acad Sci USA 1985;91:9441–5.
[41] Cominacini L, Garbin U, Pastorino AM. Increased susceptibility of LDL to in vitro oxidation in patients with insulin-dependent and non-insulin-dependent diabetes mellitus. Diab Res 1994;26:173–84.
[42] Ceriello A, Bortolotti N, Pirisi M, Crescentini A, Tonutti L, Motz E, et al. Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. Diab Care 1997;20:1589–93.
[43] Tertov VV, Sobenin IA, Kaplun VV, Orekhov AN. Antioxidant content in low density lipoprotein and lipoprotein oxidation in vivo and in vitro. Free Radic Res 1998;29:165–73.
[44] Kjaer K, Hangaard J, Petersen NE, Hagen C. Effect of simvastatin in patients with type I (insulin-dependent) diabetes mellitus and hypercholesterolemia. Acta Endocrinol (Copenh) 1992;126:229–32.
[45] van Deursen RT, Nieuwenhuijzen-Kruseman AC, Degenaar CP, Wolffenbuttel B. The effects of the cholesterol synthesis inhibitor simvastatin in patients with hypercholesterolemia with and without diabetes mellitus. Ned Tijdschr Geneeskd 1990;134:2142–6.
[46] Sobal G, Menzel EJ, Sinzinger H. Influence of acetylsalicylic acid on oxidation of native and glycated low-density lipoprotein. Life Sci 2000;66:1987–98.
[47] Sobal G, Sinzinger H, Menzel EJ. Troglitazone-binding to LDL and its glycated modifications: its role in cell-catalysed and Cu-mediated LDL-oxidation. Life Sci 2000;67:695–707.
[48] Sobal G, Menzel J, Sinzinger H. Calcium antagonists as inhibitors of in-vitro LDL-oxidation and glycation. Biochem Pharmacol 2001; 61:373–9.
[49] Liu R, Saku K, Zhang B, Hirata K, Shiomi M, Arakawa K. In vivo kinetics of oxidatively modified HDL. Biochem Med Metab Biol 1993;49:392–7.
[50] Marsche G, Hammer A, Oskolkova O, Kozarsky KF, Sattler W, Malle E. Hypochlorite-modified high density lipoprotein, a high affinity ligand to scavenger receptor class B, type I, impairs high density lipoprotein-dependent selective lipid uptake and reverse cholesterol transport. J Biol Chem 2002;277:32172–9.