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Antioxidative effect of fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, on peroxidation of phospholipid liposomes

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Abstract

The antioxidative effect of fluvastatin sodium (fluvastatin), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, on lipid peroxidation of phosphatidylcholine (PC) liposomes was investigated in various peroxidizing systems. Fluvastatin markedly inhibited the formation of thiobarbituric acid reactive substances in iron (II)-supported peroxidation of liposomes (IC50 = 1.2×10^{-5} M). The order of magnitude of inhibition of each drug on the peroxidation was: butylated hydroxytoluene > fluvastatin ⩾ probucol ⩾ pravastatin. Moreover, concentrations of fluvastatin ranging from 1×10^{-6} to 1×10^{-4} M inhibited peroxyl radical-mediated peroxidation of liposomes induced by water-soluble and lipid-soluble radical generators, 2,2′-azobis (2-amidinopropane) dihydro-chloride and 2,2′-azobis (2,4-dimethylvaleronitrile), respectively. However, pravastatin showed no effect against peroxyl radical-mediated peroxidation. These results indicate that fluvastatin acted non-enzymatically as an effective inhibitor against lipid peroxidation of PC liposomes and that the antioxidative effects of fluvastatin may be due to the scavenging action of fluvastatin on liposomal lipid peroxidation induced by peroxyl radicals generated in the aqueous and lipid phases.

Introduction

Fluvastatin is a potent, lipid-soluble inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (Tse & Labbadia 1992; Yamamoto et al 1995), and has lipid-lowering antioxidative effects (Hoffman et al 1992; Hussein et al 1997; Leonhardt et al 1997). Therefore, fluvastatin therapy may help reduce the risk of artherosclerosis and coronary heart disease (Leonhardt et al 1997; Massaeli et al 1999). Afanas'ev et al (1993) reported that the lipid-rich microsomal membranes are potential targets of injury in cells exposed to active oxygen species. The increased production of reactive oxygen species contributes to pathological processes, including membrane lipid peroxidation (Puntarulo & Cederbaum, 1996). We previously demonstrated that the suppression of both in-vivo and in-vitro lipid peroxidation in liver microsomes was due to the scavenging action of fluvastatin on free radicals, since fluvastatin chemically scavenged the active oxygen species such as hydroxyl radicals and superoxide anion (Yamamoto et al 1998). Thus, fluvastatin may be involved in antioxidant defences because the lipid-rich microsomal membranes are potential targets of injury in cells exposed to active oxygen species. However, the mechanism of the oxidative modification for membrane phospholipids is not well known.

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The aim of this work was to clarify the antioxidative effects of fluvastatin on lipid peroxidation in membrane phospholipids. Phosphatidylcholine (PC) liposomes were used as a simple model to investigate the effect of fluvastatin on lipid peroxidation induced by iron (II) or peroxyl radicals generated in the aqueous and lipid phases by the thermal decomposition of the azo-initiator 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) or the lipid-soluble 2,2′-azobis (2,4-dimethylvaleronitrile) (AMVN) (Niki 1990). The effect of the lipophilic nature of fluvastatin and antioxidants, probucol and butylated hydroxytoluene (BHT), was compared with the hydrophilic antioxidant, pravastatin, with regard to their capacity to prevent the generation of lipid peroxides in PC liposomes.

Materials and Methods

Chemicals

Fluvastatin sodium, pravastatin sodium and probucol were generous gifts from Sandoz Pharmaceuticals Ltd (Ibaraki, Japan). L-α-Phosphatidylcholine β-arachidonoyl γ-stearoyl, (PC(A)) and 2-thiobarbituric acid (TBA) were obtained from Sigma Chemical Co. (St Louis, MO) and E. Merck (Darmstadt, Germany), respectively. Phosphatidylcholine (egg yolk; PC) and azo compounds AAPH and AMVN were purchased from Wako Pure Chemicals (Osaka, Japan). *cis*-Parinaric acid (PnA) was purchased from Calbiochem-Navabiochem Co. (La Jolla, CA). Other reagents were of analytical grade.

Preparation of phospholipid liposomes

PC liposomes using an iron (II)-supported peroxidation system were prepared by the method of Fukuzawa et al (1981) with a minor modification. PC (15.4 mg) was dissolved in 4 mL chloroform, evaporated to dryness under nitrogen, and dispersed in 10 mL 0.1 M Tris-HCl buffer, pH 7.4. PC(A) liposomes using the AAPH-induced peroxidation system were prepared by the method of Tampo & Yonaha (1991). PC(A) (3.5 mg) was dissolved in 1 mL chloroform, the solvent removed under Ar gas, and dispersed in 1 mL 0.1 M Tris-HCl buffer containing 0.25 M NaCl, pH 7.4. PC(A) liposomes using the AMVN-induced peroxidation system were prepared by the method of Custodio et al (1994). PC(A) (3.5 mg) with AMVN (0.41 mg) was dissolved in 1 mL chloroform, the solvent removed under Ar gas, and dispersed in 1 mL 20 mM Tris-HCl buffer, pH 7.4, in a water bath at 35°C with hand-shaking. All liposomal suspensions were sonicated in ice-cold water and were stored under nitrogen at 0°C.

Lipid peroxidation supported by iron (II) in PC liposomes

Lipid peroxidation supported by iron (II) in PC liposomes was estimated by monitoring the thiobarbituric acid reactive substances formation according to the method of Afanas'ev et al (1989). PC liposomes (0.03 mg mL^{-1}) were incubated at 25°C for 60 min with 0.1 mM $FeSO_4$, with or without each drug ($7.5 \times 10^{-9} - 5.0 \times 10^{-4}$ M) and 0.1 M Tris-HCl buffer, pH 7.4. Lipid peroxidation was terminated by adding 0.5 mL 0.5 M trichloroacetic acid and 0.2 mL 0.1 M EDTA. Then 1 mL 0.8% (w/v) TBA was added and the solution was heated at 100°C for 15 min. After centrifugation of precipitated proteins, the malondialdehyde content was determined by measuring the absorbance at 532 nm ($\epsilon = 1.56 \times 10^5$ M^{-1} cm^{-1}), according to the method of Buege & Aust (1978).

Lipid peroxidation induced by water-soluble peroxyl radical

The membrane lipid peroxidation induced by peroxyl radicals generated in the aqueous phase by thermal decomposition of the azo-initiator AAPH was kinetically followed by the measurement of oxygen consumption using an Oxygen Monitor Model OBH-100 (Otsuka Electronics Co., Osaka, Japan.) (Takenaka et al 1991). The reaction mixture (1 mL) consisted of 200 μM PC(A) liposomes in 20 mM phosphate buffer containing 100 mM NaCl, pH 7.0. The oxidation was carried out in a thermostated glass cuvette at 37°C, provided with magnetic stirring and initiated by addition of 10 mM AAPH (Laranjinha et al 1992). The concentrations of fluvastatin and pravastatin were preincubated with liposomes for 20 min at 37°C before the addition of AAPH. Blank experiments in the absence of PC(A) liposome were performed to evaluate the oxygen consumption induced by 10 mM AAPH.

Lipid peroxidation induced by lipid-soluble peroxyl radical

The oxidative degradation of PnA (7.5 μM) incorporated in liposomes (800 μM PC(A)) containing AMVN (330 μM) was induced by peroxyl radicals generated by thermal decomposition of the lipid-soluble AMVN at 50°C (Kuypers 1987; Kagan et al 1993). Each con-

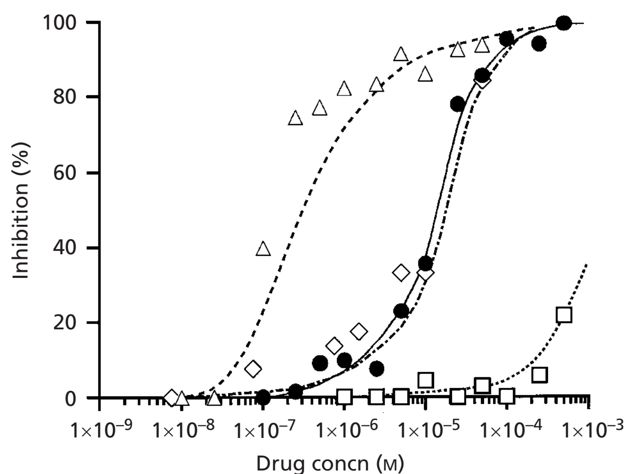

Figure 1 Inhibition of the formation of thiobarbituric acid reactive substances in iron (II)-supported peroxidation of phospholipid liposomes by fluvastatin (●), pravastatin (□), probucol (◇) and butylated hydroxytoluene (△). The formation of thiobarbituric acid reactive substances was evaluated in terms of nmol malondialdehyde and expressed as a percentage inhibition from the control level (in the absence of the drugs). A typical control value was 30.1 nmol mg^{-1} PC h^{-1}.

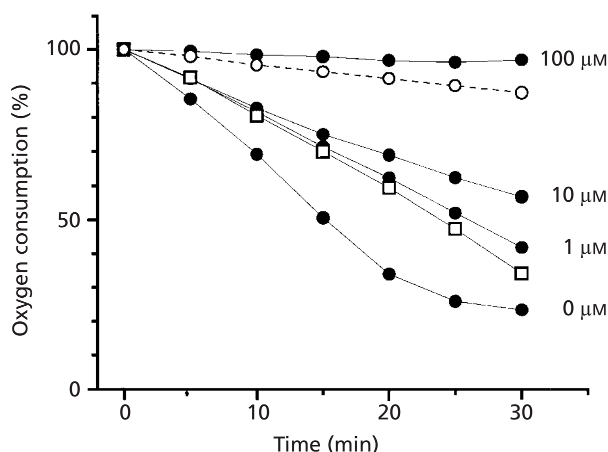

Figure 2 Inhibition of oxygen consumption by fluvastatin and pravastatin in 2,2′-azobis (2-amidinopropane) dihydro-chloride (AAPH)-induced peroxidizing system containing phospholipid liposomes. Each concentration of fluvastatin (●) and 100 μM pravastatin (□) was preincubated with liposomes before the addition of AAPH. The oxygen consumption induced by AAPH in the absence of liposomes is shown (○). A typical example of the three independent experiments is shown.

centration of fluvastatin and pravastatin was preincubated with liposomes for 20 min at 37°C before starting the PnA assay. The incorporation of PnA was achieved by incubating a sample of an ethanolic solution of 750 μM PnA with liposomes containing each drug for 2.5 min at 37°C. PnA degradation was monitored by detecting the decay of fluorescence in a Fluorescence Spectrometer Model F-2000 (Hitachi Co., Ibaraki, Japan) provided with thermostated cuvettes and magnetic stirring. The excitation and the emission wavelengths were set at 324 and 413 nm, respectively. The excitation and emission slit widths were 5 nm. Blank experiments using liposomes prepared without AMVN were performed to evaluate the spontaneous fluorescence decay of PnA.

Results

Inhibition of iron (II)-supported peroxidation of liposomes

We investigated the inhibitory effect of fluvastatin on the non-enzymatic peroxidizing system using liposomes. Figure 1 shows the inhibition of fluvastatin, pravastatin, probucol and BHT on lipid peroxidation supported by iron (II) in liposomes. The lipid peroxidation was estimated by thiobarbituric acid reactive substances formation. Concentrations of fluvastatin ($1.0\times10^{-7}-5.0\times10^{-4}$ M), pravastatin ($1.0\times10^{-6}-5.0\times10^{-4}$ M), BHT ($1.0\times10^{-8}-5.0\times10^{-5}$ M) and probucol ($7.5\times10^{-9}-5.0\times10^{-5}$ M) were added to the incubation mixture in the presence of liposomes and 0.1 mM $FeSO_4$. The IC50 of fluvastatin, BHT and probucol was 1.2×10^{-5}, 3.0×10^{-7} and 2.3×10^{-5} M, respectively (Figure 1). These compounds inhibited lipid peroxidation in the following order of potency: BHT $>$ fluvastatin $\geqslant$ probucol $\gg$ pravastatin.

Inhibition of oxygen consumption in AAPH-induced peroxidizing system containing phospholipid liposomes by fluvastatin or pravastatin

AAPH, a water-soluble radical generator, produces peroxyl radicals in the aqueous phase, and the peroxyl radicals attack phospholipids at the membrane surface (Niki 1990). Figure 2 shows the effect of fluvastatin and pravastatin on AAPH-induced lipid peroxidation of liposomes. Liposomes with AAPH, which generates initiating radicals at a constant rate in the aqueous phase, caused a marked oxygen consumption by approximately 80% when measured at 30 min after the addition of AAPH (Figure 2). Fluvastatin at concentrations ranging from 1 to 100 μM suppressed

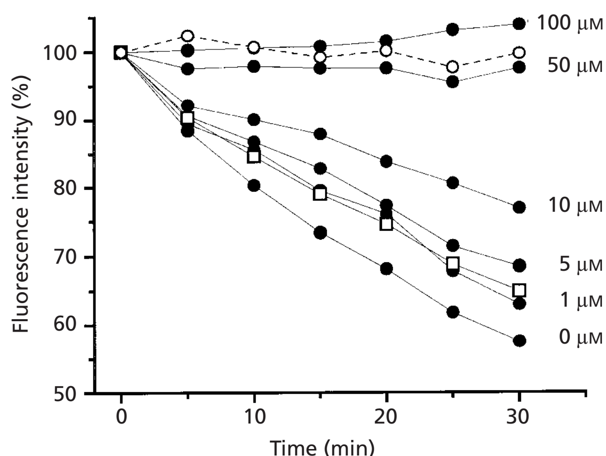

Figure 3 Inhibition of 2,2′-azobis (2,4-dimethylvaleronitrile) (AMVN)-induced PnA fluorescence decay by fluvastatin and pravastatin in phospholipid liposomes. Fluvastatin (●) and 100 μM pravastatin (□) were preincubated with liposomes before the PnA assay. The spontaneous decay of fluorescence of PnA at 50°C without AMVN is shown (○). A typical example of the three independent experiments is shown.

the AAPH-induced oxygen consumption in a dose-dependent manner. However, 100 μM pravastatin inhibited oxygen consumption to a lesser extent than 1 μM fluvastatin. In this study, 1 mM pravastatin did not inhibit the AAPH-induced oxygen consumption (data not shown).

Inhibition of AMVN-induced PnA fluorescence decay by fluvastatin and pravastatin in phospholipid liposomes

PnA as a polyunsaturated fatty acid has been used to determine the susceptibility of membranes to lipid peroxidation and to study the effects of drugs of pharmacological interest on membrane peroxidation (Kuypers et al 1987; van den Berg et al 1988, 1991; Kagan et al 1993). Figure 3 shows the effect of fluvastatin and pravastatin on lipid peroxidation induced by AMVN, a lipid-soluble radical generator, as monitored by the decrease in fluorescence intensity of PnA. In the absence of AMVN, the fluorescence intensity of PnA remained unchanged during the measurement. In the absence of fluvastatin, the fluorescence of the PnA decreased by approximately 60 % in 30 min (Figure 3). Fluvastatin at concentrations ranging from 1 to 100 μM inhibited the AMVN-induced decay of fluorescence of PnA dose-dependently. Pravastatin at a concentration of 100 μM showed the same effect on the fluorescence decay of PnA as 1 μM fluvastatin. Moreover, 1 mM pravastatin did not affect the AMVN-induced decay of fluorescence of PnA (data not shown).

Discussion

We previously reported that fluvastatin, which had no effect on the content of cytochrome P450 and the activity of NADPH-cytochrome P450 reductase in liver microsomes, markedly inhibited NADPH-dependent lipid peroxidation, and this suppression might be due to the scavenging action of fluvastatin on free radicals (Yamamoto et al 1998). In this study, to clarify the mechanism of the antioxidative effects of fluvastatin on membrane lipid peroxidation, we investigated whether the antioxidative action of fluvastatin occurs non-enzymatically, that is directly to membrane phospholipids. We examined the inhibitory effect of fluvastatin on iron (II)-induced PC liposomal peroxidation by monitoring the thiobarbituric acid reactive substances formation. PC liposomes are frequently used as a simple model for cellular and subcellular biomembranes (Murase et al 1997, 1998). In our experiment, fluvastatin markedly inhibited the formation of thiobarbituric acid reactive substances in iron (II)-supported peroxidation of liposomes (Figure 1).

Probucol and BHT are also highly lipophilic, and the antioxidant property of both drugs is partly due to their free radical scavenging effect (Thompson et al 1989; Valoti et al 1989; Hiramatsu et al 1994). Pravastatin, the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is similar in structure to fluvastatin. However, pravastatin possesses the hydrophilicity of the β-hydroxy group at the 6 position of decaline which contributes to the tissue-selectivity (Koga et al 1990) and is highly hydrophobic. Fluvastatin has a 4-fluorophenyl group at the 3 position of indole. The double bond conjugated with the indole ring plays an important role for the antioxidative activity of fluvastatin (Suzumura et al 1999; Nakamura et al 2000) and fluvastatin is both lipophilic and hydrophilic. In this study, the inhibitory effect of fluvastatin was similar to that of probucol, and was more potent than that of pravastatin, but less potent than that of BHT (Figure 1). In addition, the IC50 value and inhibitory magnitude of each drug on liposomal lipid peroxidation agreed with our results on microsomal peroxidation (Yamamoto et al 1998).

Lipid peroxidation is a free radical-mediated chain reaction forming lipid hydroperoxides occurring in the unsaturated fatty acids of membrane phospholipid (Esterbauer & Cheeseman 1990). The peroxidation of liposomal phospholipids is responsible for chain-carrying lipid peroxyl radicals (Lim et al 1992). We investigated whether fluvastatin acts as an effective inhibitor of lipid peroxidation in PC liposomes induced by water-soluble and lipid-soluble radical generators, AAPH and AMVN, respectively. The addition of the water-soluble AAPH generates free radicals outside the phospholipid membrane and these radicals attack the liposomal membrane at the membrane surface (Murase et al 1997, 1998). In AAPH-induced oxidation, fluvastatin added to the aqueous phase suppressed the free radical chain-reaction efficiently and inhibited the lipid peroxidation of the liposomal membrane (Figure 2), suggesting that fluvastatin is an efficient antioxidant against radicals generated in the aqueous phase. In contrast, a lipid-soluble AMVN incorporated in liposomes generates free radicals within a lipid bilayer (Murase et al 1998). Fluvastatin is lipid-soluble and can easily be incorporated into the lipid bilayer (Yamamoto et al 1995, 1998). In AMVN-induced oxidation, fluvastatin incorporated in the liposomal membrane inhibited membrane lipid peroxidation (Figures 2 and 3). In these systems, it would seem that the chain-initiating peroxyl radicals are generated from AMVN and that chain-propagation reactions would proceed within the membranes (Niki 1990; Massaeli et al 1999). In fact, lipid peroxidation was not observed when PC liposomes containing AMVN were preincubated with fluvastatin (data not shown). Fluvastatin is slightly lipophilic, whereas pravastatin is highly hydrophilic. Therefore, we presumed that fluvastatin is located both outside and inside of the liposomal membrane and can scavenge both the chain-initiating and chain-propagating radicals in the aqueous and lipid phases. This may indicate that fluvastatin operates most effectively in lipid-rich biomembranes to suppress the initiation or propagation of lipid peroxidation, but pravastatin operates only slightly because of its hydrophilicity.

These results suggest that fluvastatin acts as an antioxidant by scavenging radicals generated in both the chain-initiating radicals in the aqueous phase (i.e. a water-soluble peroxyl radical) and the chain-propagating radicals in the lipid phase (i.e. a lipid-soluble peroxyl radical). Fluvastatin may prevent the initiation and promotion of biomembrane lipids by the mechanisms scavenging free radicals and may offer significant protection against atherosclerotic heart disease.

References

Afanas'ev, I. B., Dorozhko, A. I., Brodskii, A. V., Kostyuk, V. A., Potapovitch, A. I. (1989) Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol.* **38**: 1763–1769

Afanas'ev, I. B., Dorozhko, A. I., Polozova, N. I., Kuprianova, N. S., Brodskii, A. V., Ostrachovitch, E. A., Korkina, L. G. (1993) Is superoxide an initiator of microsomal lipid peroxidation? *Arch. Biochem. Biophys.* **302**: 200–205

Buege, J. A., Aust, S. D. (1978) Microsomal lipid peroxidation. *Methods Enzymol.* **52**: 302–310

Custodio, J. B., Dinis, T. C., Almeida, L. M., Madeira, V. M. (1994) Tamoxifen and hydroxytamoxifen as intramembraneous inhibitors of lipid peroxidation. Evidence for peroxyl radical scavenging activity. *Biochem. Pharmacol.* **47**: 1989–1998

Esterbauer, H., Cheeseman, K. H. (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods Enzymol.* **186**: 407–421

Fukuzawa, K., Chida, H., Tokumura, A., Tsukatani, H. (1981) Antioxidative effect of alpha-tocopherol incorporation into lecithin liposomes on ascorbic acid-Fe2+-induced lipid peroxidation. *Arch. Biochem. Biophys.* **206**: 173–180

Hiramatsu, M., Liu, J., Edamatsu, R., Ohba, S., Kadowaki, D., Mori, A. (1994) Probucol scavenged 1,1-diphenyl-2-picrylhydrazyl radicals and inhibited formation of thiobarbituric acid reactive substances. *Free Radic. Biol. Med.* **16**: 201–206

Hoffman, R., Brook, G. J., Aviram, M. (1992) Hypolipidemic drugs reduce lipoprotein susceptibility to undergo lipid peroxidation: in vitro and ex vivo studies. *Atherosclerosis* **93**: 105–113

Hussein, O., Schlezinger, S., Rosenblat, M., Keidar, S., Aviram, M. (1997) Reduced susceptibility of low density lipoprotein (LDL) to lipid peroxidation after fluvastatin therapy is associated with the hypocholesterolemic effect of the drug and its binding to the LDL. *Atherosclerosis* **128**: 11–18

Koga, T., Simada, Y., Kuroda, M., Tsujita, Y., Hasegawa, K., Yamazaki, M. (1990) Tissue-selective inhibition of cholesterol synthesis in vivo by pravastatin sodium, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Biochim. Biophys. Acta* **1045**: 115–120

Kagan, V. E., Tsuchiya, M., Serbinova, E., Packer, L., Sies, H. (1993) Interaction of the pyridoindole stobadine with peroxyl, superoxide and chromanoxyl radicals. *Biochem. Pharmacol.* **45**: 393–400

Kuypers, F. A., van den Berg, J. J., Schalkwijk, C., Roelofsen, B., Op den Kamp, J. A. (1987) Parinaric acid as a sensitive fluorescent probe for the determination of lipid peroxidation. *Biochim. Biophys. Acta* **921**: 266–274

Laranjinha, J. A., Almeida, L. M., Madeira, V. M. (1992)

Lipid peroxidation and its inhibition in low density lipoproteins: quenching of cis-parinaric acid fluorescence. *Arch. Biochem. Biophys.* **297**: 147–154

Leonhardt, W., Kurktschiev, T., Meissner, D., Lattke, P., Abletshauser, C., Weidinger, G., Jaross, W., Hanefeld, M. (1997) Effects of fluvastatin therapy on lipids, antioxidants, oxidation of low density lipoproteins and trace metals. *Eur. J. Clin. Pharmacol.* **53**: 65–69

Lim, B. P., Nagao, A., Terao, J., Tanaka, K., Suzuki, T., Takama, K. (1992) Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. *Biochim. Biophys. Acta* **1126**: 178–184

Massaeli, H., Sobrattee, S., Pierce, G. N. (1999) The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein peroxidation. *Free Radic. Biol. Med.* **26**: 1524–1530

Murase, H., Yamauchi, R., Kato, K., Kunieda, T., Terao, J. (1997) Synthesis of a novel vitamin E derivative, 2-(alpha-D-glucopyranosyl) methyl-2,5,7,8-tetramethylchroman-6-ol, by alpha-glucosidase-catalyzed transglycosylation. *Lipids* **32**: 73–78

Murase, H., Moon, J. H., Yamauchi, R., Kato, K., Kunieda, T., Yoshikawa, T., Terao, J. (1998) Antioxidant activity of a novel vitamin E derivative, 2-(alpha-D glucopyranosyl) methyl-2,5,7,8-tetramethylchroman-6-ol. *Free Radic. Biol. Med.* **24**: 217–225

Nakamura, T., Nishi, H., Kokusenya, Y., Hirota, K., Miura, Y. (2000) Mechanism of antioxidative activity of fluvastatin–determination of the active position. *Chem. Pharm. Bull.* **48**: 235–237

Niki, E. (1990) Free radical initiators as source of water- or lipid-soluble peroxyl radicals. *Methods Enzymol.* **186**: 100–108

Puntarulo, S., Cederbaum, A. I. (1996) Role of cytochrome P-450 in the stimulation of microsomal production of reactive oxygen species by ferritin. *Biochim. Biophys. Acta* **1289**: 238–246

Suzumura, K., Yasuhara, M., Tanaka, K., Suzuki, T. (1999) Protective effect of fluvastatin sodium (XU-62-320), a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on oxidative modification of human low-density lipoprotein in vitro. *Biochem. Pharmacol.* **57**: 697–703

Takenaka, Y., Miki, M., Yasuda, H., Mino, M. (1991) The effect of alpha-tocopherol as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. *Arch. Biochem. Biophys.* **285**: 344–350

Tampo, Y., Yonaha, M. (1991) Mechanism of cobalt (II) ion inhibition of iron-supported phospholipid peroxidation. *Arch. Biochem. Biophys.* **289**: 26–32

Thompson, D. C., Cha, Y. N., Trush, M. A. (1989) The peroxidase-dependent activation of butylated hydroxyanisole and butylated hydroxytoluene (BHT) to reactive intermediates. Formation of BHT-quinone methide via a chemical-chemical interaction. *J. Biol. Chem.* **264**: 3957–3965

Tse, F. L., Labbadia, D. (1992) Absorption and disposition of fluvastatin, an inhibitor of HMG-CoA reductase, in the rabbit. *Biopharm. Drug Dispos.* **13**: 285–294

Valoti, M., Sipe, H. J., Sgaragli, G., Mason, R. P. (1989) Free radical intermediates during peroxidase oxidation of 2-t-butyl-4-methoxyphenol, 2,6-di-t-butyl-4-methylphenol, and related phenol compounds. *Arch. Biochem. Biophys.* **269**: 423–432

van den Berg, J. J., Kuypers, F. A., Qju, J. H., Chiu, D., Lubin, B., Roelofsen, B., Op den Kamp, J. A. (1988) The use of cis-parinaric acid to determine lipid peroxidation in human erythrocyte membranes. Comparison of normal and sickle erythrocyte membranes. *Biochim. Biophys. Acta* **944**: 29–39

van den Berg, J. J., Kuypers, F. A., Lubin, B. H., Roelofsen, B., Op den Kamp, J. A. (1991) Direct and continuous measurement of hydroperoxide-induced oxidative stress on the membrane of intact erythrocytes. *Free Radic. Biol. Med.* **11**: 255–261

Yamamoto, A., Itoh, S., Hoshi, K., Ichihara, K. (1995) Inhibition of cholesterol synthesis ex vivo and in vivo by fluvastatin, a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Experientia* **51**: 223–226

Yamamoto, A., Hoshi, K., Ichihara, K. (1998) Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsomes. *Eur. J. Pharmacol.* **361**: 143–149