ELSEVIER

European Journal of Pharmacology 361 (1998) 143–149

ejp

Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsomes

Atsuko Yamamoto, Katsuji Hoshi *, Kazuo Ichihara

Department of Pharmacology, Hokkaido College of Pharmacy, 7-1 Katsuraoka, 047-0264 Otaru, Japan

Received 4 May 1998; revised 16 September 1998; accepted 18 September 1998

Abstract

We investigated the effect of fluvastatin sodium (fluvastatin) and pravastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, on the formation of thiobarbituric acid reactive substances both in vivo and in vitro in rat liver microsomes and on active oxygen species. Oral administration of fluvastatin at low doses (3.13 and 6.25 mg/kg) inhibited the formation of thiobarbituric acid reactive substances in rat liver microsomes, but high doses (12.5 and 25 mg/kg) did not change the formation of thiobarbituric acid reactive substances. Fluvastatin at any dose used had no effect on the content of cytochrome P-450 and the activity of NADPH-cytochrome P-450 reductase. In in vitro experiments, concentrations of fluvastatin ranging from 1×10^{-6}–1×10^{-4} M markedly inhibited NADPH-dependent lipid peroxidation in liver microsomes, but pravastatin weakly inhibited lipid peroxidation. The order of magnitude of inhibition of each drug on in vitro lipid peroxidation was butylated hydroxytoluene > probucol ≥ fluvastatin > pravastatin. Moreover, fluvastatin chemically scavenged active oxygen species such as hydroxyl radicals and superoxide anion generated by the Fenton reaction and by the xanthine–xanthine oxidase system, respectively, but pravastatin showed no scavenging of superoxide anion. These results indicate that the suppression of in vivo and in vitro lipid peroxidation in liver microsomes may be, at least in part, due to the scavenging by fluvastatin of free radicals. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: HMG-CoA reductase inhibitor; Fluvastatin; Lipid peroxidation; Free radical scavenger

1. Introduction

Fluvastatin is a new and potent inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis. Cholesterol synthesis in the liver, the major organ supplying serum cholesterol, is regulated by cellular HMG-CoA reductase and low-density lipoprotein (LDL) receptors. Hussein et al. (1997) have demonstrated that fluvastatin reduces the LDL level in plasma. The oxidative modification of the LDL level in plasma or tissue is related to the progression of atherosclerotic disease (Rudling and Collins, 1996; Rifici and Khachadurian, 1996). The antiatherogenic properties of fluvastatin are related to its ability to reduce the susceptibility of LDL to lipid peroxidation (Hussein et al., 1997). There is evidence that LDL oxidation occurs in vivo (Rifici and Khachadurian, 1996), but the mechanism for this is not well known.

Recently, we also reported that fluvastatin was more potent than pravastatin in inhibiting both ex vivo and in vivo sterol synthesis in rat liver (Yamamoto et al., 1995). Transon et al. (1995, 1996) described that fluvastatin competitively inhibited cytochrome $P\text{-}450_{TB}$ (CYP2C9) in vivo and in vitro. Cytochrome P-450 in hepatic microsomes is believed to be predominant in causing oxidative damage, that is, radical species (Ingelman-Sundberg, 1986; Hu et al., 1994; Bestervelt et al., 1995). In this connection, 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 and Cederbaum, 1996).

* Corresponding author. Tel.: +81-134-62-1858; Fax: +81-134-62-1858; E-mail: khoshi@hucc.hokudai.ac.jp

0014-2999/98/$ - see front matter © 1998 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(98)00692-X

The present study, therefore, was undertaken to examine the influence of fluvastatin on both drug-metabolizing enzymes and lipid peroxidation in rat liver microsomes. Moreover, the in vitro effects of fluvastatin on lipid peroxidation were also included for comparison with the effects of another HMG-CoA reductase inhibitor, pravastatin, and the antioxidant agents, butylated hydroxytoluene and probucol. This study was undertaken to examine the hydroxyl radical and superoxide scavenging activity of fluvastatin, using both electron spin resonance (ESR) spectrometry and chemiluminescence.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (7 weeks old, 250–300 g body weight) were obtained from Clea Japan, Tokyo. The animal room was maintained at 23 ± 1°C, with 50 ± 5% relative humidity and a 12-h light–dark cycle (lights on 6:00 to 18:00). The rats were given water and commercial laboratory chow (MF; Oriental Yeast, Japan) ad libitum for at least one week before use.

2.2. Materials

Fluvastatin sodium, pravastatin sodium and probucol were generous gifts from Sandoz Pharmaceuticals (Ibaraki, Japan). NADPH and 2-thiobarbituric acid were obtained from Oriental Yeast (Tokyo, Japan) and E. Merck (Darmstadt, Germany), respectively. The spin-trapping agent, 5,5-dimethylpyrroline-*N*-oxide (DMPO), was purchased from Sigma (St. Louis, MO). 2-Methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Other reagents were of analytical grade.

2.3. Treatment of animals and preparation of microsomes

Rats were orally given fluvastatin at single doses of 3.13, 6.25, 12.5 and 25.0 mg/kg, respectively. The control animals received an equivalent volume of water.

Animals were killed by decapitation 0.25–24 h after acute administration of the drug, and the liver was rapidly removed. The liver microsomal fraction was prepared from a 20% homogenate in 50 mM Tris–HCl buffer (pH 7.4), as reported previously (Hoshi et al., 1989). All assays were carried out on the same day that the tissue was prepared.

2.4. Determination of drug-metabolizing enzymes

The cytochrome P-450 content and the NADPH-cytochrome P-450 reductase (fp_2) activity in rat liver microsomes were measured by the method of Omura and Sato (1964) and Phillips and Langdon (1962), respectively. Microsomal protein was determined using the method of Lowry et al. (1951).

2.5. Determination of lipid peroxidation products

In in vivo experiments, preparation of rat liver microsomes for lipid peroxidation was carried out under a stream of nitrogen. Malondialdehyde levels were determined by monitoring thiobarbituric acid reactive substances according to the method of Ohkawa et al. (1979) with minor modifications. The reaction mixture contained 0.1 ml of microsomes (approximately 20 mg protein/ml) in 50 mM Tris–HCl buffer (pH 7.4), 0.2 ml of 0.8% sodium dodecylsulfate, 1.5 ml of 20% acetic acid solution (pH 3.5) and 0.5% aqueous solution of thiobarbituric acid. The mixture was heated at 95°C for 60 min. After cooling with tap water, the mixture was extracted with *n*-butanol and the absorbance of the organic layer was measured at 532 nm. 1,1,3,3-Tetraethoxypropane was used as an external standard, and the level of thiobarbituric acid reactive substances was expressed as nmol of malondialdehyde.

In the in vitro experiments, microsomes were prepared from the livers of untreated rats. Microsomal lipid peroxidation was measured with the NADPH-dependent iron pyrophosphate system according to Davis et al. (1987) with minor modifications. The incubation mixture contained 0.5 mg microsomal protein per ml, 0.15 M KCl, 50 μM ferric pyrophosphate, and each drug (7.5×10^{-8}–1×10^{-2} M). The drugs were dissolved in 25 mM Tris–HCl buffer (pH 7.4). The reactions were initiated by the addition of 0.5 mM NADPH after a 2-min temperature equilibrium period. Aliquots removed at 5 min for malondialdehyde measurements were added to 30% trichloroacetic acid and allowed to react with 0.75% thiobarbituric acid reagent at 95°C for 20 min, according to the method of Ottolenghi (1959). The color complex was determined spectrophotometrically by using the extinction coefficient at 535 nm of 156 mM^{-1} cm^{-1} (Jordan and Schenkman, 1982).

2.6. Hydroxyl radical analysis

The experiment was carried out at pH 7.0 at 25°C. The final concentrations of mixture were set at 200 μM H_2O_2, 100 μM $FeCl_2$, 1 mM DMPO, 50 mM potassium phosphate buffer (pH 7.0) and 0.01–1 mM of fluvastatin or pravastatin. The concentration of DMPO spin adducts of hydroxyl radicals (DMPO-OH) formed was recorded 60 s after the addition of DMPO. The signal intensities were evaluated as the peak height of the second signal of the quartet of DMPO-OH spin adducts. Electron spin resonance (ESR) spectra were measured at 25°C with a JEOL JES-TE300 ESR spectrometer (Tokyo, Japan). Typical ESR settings were field, 3350 ± 50 G; frequency, 9.42 GHz; modulation, 100 kHz × 1 G; sweep time, 4 min.

Table 1
Effects of a single administration of fluvastatin on hepatic microsomal enzymes responsible for drug metabolism

Treatment	mg/kg	P-450 content (nmol/mg protein)	fp_2 activity (nmol/min/mg protein)
Control		0.87 ± 0.05	179.5 ± 9.5
Fluvastatin	3.13	0.77 ± 0.01	154.3 ± 8.6
	6.25	0.89 ± 0.06	183.1 ± 14.0
	12.5	0.97 ± 0.03	176.3 ± 16.7
	25.0	0.90 ± 0.07	191.8 ± 21.9

Rats were orally given fluvastatin at a single dose of 3.13, 6.25, 12.5 and 25.0 mg/kg, respectively. The control animals received an equivalent volume of water. The content of cytochrome P-450 (P-450) and the activity of NADPH-cytochrome P-450 reductase (fp_2) in liver microsome were measured 1 h after drug administration. Each value represents the mean ± S.E.M. for 6–8 rats.

2.7. Superoxide analysis

Superoxide was generated by the xanthine–xanthine oxidase system. The final concentrations of mixture were set at 100 μM xanthine, 5 μM 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA), 0.08 μM catalase and 0.1–2 mM of fluvastatin or pravastatin. The direct superoxide radical scavenging activity of fluvastatin or pravastatin was measured by the chemiluminescence method (Gotoh and Niki, 1994).

2.8. Statistical analysis

Data were analyzed for significance by the Student–Newman–Keuls test. Values are given as means ± S.E.M. Differences at a level < 0.05 were considered significant. The concentration required to produce a 50% inhibition (IC_{50}) of each drug was calculated by log-probit regression analysis.

3. Results

3.1. Effects of fluvastatin on drug-metabolizing enzymes in microsomes

We first studied the effects of fluvastatin on the activity of the drug-metabolizing enzymes. The content of cytochrome P-450 and the activity of fp_2 were not changed at 1 h after single administration of a 3.13–25.0 mg/kg dose of fluvastatin (Table 1). Moreover, fluvastatin at any dose used had no effect on drug metabolism at 0.25–24 h (data not shown).

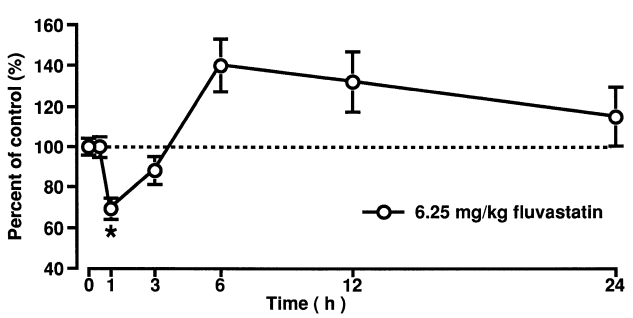

Fig. 1. Time course of inhibition by fluvastatin of microsomal lipid peroxidation in rat liver. The inhibition of lipid peroxidation was measured after oral administration of 6.25 mg/kg of fluvastatin. The formation of thiobarbituric acid reactive substances in microsomes is expressed in terms of nmol malondialdehyde, using tetraethoxypropane as a standard. Each point represents the mean ± S.E.M. for 6–8 rats. $* P < 0.05$ vs. control.

3.2. Effects of fluvastatin on in vivo lipid peroxidation in microsomes

Fig. 1 illustrates the time course of the inhibition of thiobarbituric acid reactive substances produced by 6.25 mg/kg of fluvastatin. Fluvastatin produced a significant decrease in lipid peroxidation to 69% 1 h after its administration, but peroxidation returned approximately to the control levels at 3 h, and subsequently this level did not change until 24 h after fluvastatin administration. Table 2 shows the inhibition of lipid peroxidation 1 h after the administration of fluvastatin concentrations ranging from 3.13 to 25.0 mg/kg. Low doses (3.13 and 6.25 mg/kg) of fluvastatin significantly inhibited lipid peroxidation in hepatic microsomes. However, lipid peroxidation was not affected by high doses (12.5 and 25.0 mg/kg) of fluvastatin.

3.3. Effects of fluvastatin on in vitro lipid peroxidation in microsomes

In vitro experiments were carried out to determine the direct effect of fluvastatin and related compounds on lipid

Table 2
Effects of a single administration of fluvastatin on microsomal lipid peroxidation in rat liver

Treatment	mg/kg	Formation of TBARS (percentage of control)
Control		100.0
Fluvastatin	3.13	74.9 ± 7.4*
	6.25	69.1 ± 4.1*
	12.5	85.4 ± 6.3
	25.0	95.3 ± 7.6

Rats were orally given fluvastatin at a single dose of 3.13, 6.25, 12.5 and 25.0 mg/kg, respectively. The control animals received an equivalent volume of water. The formation of thiobarbituric acid reactive substances (TBARS) in microsomes is expressed in terms of nmol malondialdehyde, using tetraethoxypropane as a standard. Each value represents the mean ± S.E.M. for 6–8 rats. $* P < 0.05$ vs. control.

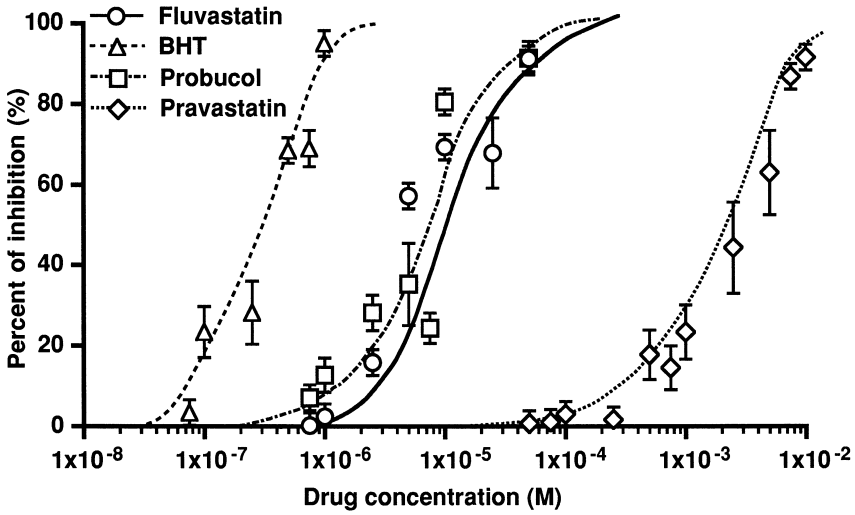

Fig. 2. Effects of fluvastatin, pravastatin, butylated hydroxytoluene (BHT) or probucol on in vitro lipid peroxidation in rat liver microsomes. Concentrations (7.5×10^{-8}–1.0×10^{-2} M) of each drug were added to the incubation mixture in the presence of microsomes. The formation of thiobarbituric acid reactive substances in microsomes is expressed in terms of nmol malondialdehyde, using tetraethoxypropane as a standard. Each point represents the mean ± S.E.M. from 6–10 experiments performed in duplicate.

peroxidation. As shown in Fig. 2, fluvastatin (7.5×10^{-7}–5.0×10^{-5} M), pravastatin (5.0×10^{-5}–1.0×10^{-2} M), butylated hydroxytoluene (7.5×10^{-8}–1.0×10^{-6} M) and probucol (7.5×10^{-7}–5.0×10^{-5} M) were added to the incubation mixture in the presence of liver microsomes as an enzyme source. Fluvastatin was found to inhibit lipid peroxidation. These compounds caused the inhibition of lipid peroxidation in the following order of potency: butylated hydroxytoluene > probucol ≥ fluvastatin > pravastatin. The inhibitory effect of fluvastatin was similar to that of probucol, and was more potent than that of pravastatin, but less than that of butylated hydroxytoluene. In addition, the concentration required to produce a 50% inhibition was calculated by log-probit regression analysis. The IC_{50} values of fluvastatin, butylated hydroxytoluene, probucol and pravastatin were 11.8×10^{-6}, 0.3×10^{-6}, 8.2×10^{-6} and 2.3×10^{-3}, respectively (Table 3).

3.4. Hydroxyl radical scavenging activity

The direct hydroxyl radical scavenging activity of fluvastatin or pravastatin was determined by ESR studies. The hydroxyl radical generated by the Fenton reaction reacted with DMPO to yield the typical DMPO-OH spin adduct spectrum (1:2:2:1 quartet), with identical hyperfine splitting constants $a\mathrm{N} = a\mathrm{H} = 14.9$ G (Finkelstein et al., 1980) (Fig. 3, A). The addition of fluvastatin (Fig. 3, B_1–B_4) and pravastatin (Fig. 3, C_1–C_4) inhibited dose dependently DMPO-OH formation. With fluvastasin, the control signal height intensity was reduced by 16% at 0.01 mM, 48% at 0.1 mM, 72% at 0.5 mM and 80% at 1 mM ($IC_{50} = 0.12$ mM, correlation coefficient = 0.99). With pravastatin, the control signal height intensity was reduced by 0% at 0.01 mM, 25% at 0.1 mM, 74% at 0.5 mM and 91% at 1 mM ($IC_{50} = 0.17$ mM, correlation coefficient = 0.97).

3.5. Superoxide scavenging activity

The direct superoxide radical scavenging activity of fluvastatin or pravastatin was measured by the chemiluminescence method. The superoxide generated by the xanthine–xanthine oxidase system reacted with CLA to emit chemiluminescence at 380 nm. Fluvastatin decreased the chemiluminescence intensity of superoxide in a dose-dependent manner (Fig. 4, A), but pravastatin did not affect it at the same dosages (data not shown). The plot of I_o/I_x as a function of [X]/[CLA] gave a straight line (Fig. 4, B). By using the result and the reported rate constants $k = 1.06 \times 10^5$ M^{-1} s^{-1} (Gotoh and Niki, 1992) for the reactions of superoxide with CLA, the rate constants for the reactions of superoxide with fluvastatin were calculated as $k = 4.2 \times 10^2$ M^{-1} s^{-1} at 25°C.

Table 3
IC_{50} values for inhibition of in vitro lipid peroxidation by fluvastatin, butylated hydroxytoluene (BHT), probucol or pravastatin in rat liver microsomes

Treatment	IC_{50}(M)
Fluvastatin	11.8×10^{-6}
BHT	0.3×10^{-6}
Probucol	8.2×10^{-6}
Pravastatin	2.3×10^{-3}

These date are shown in Fig. 2.

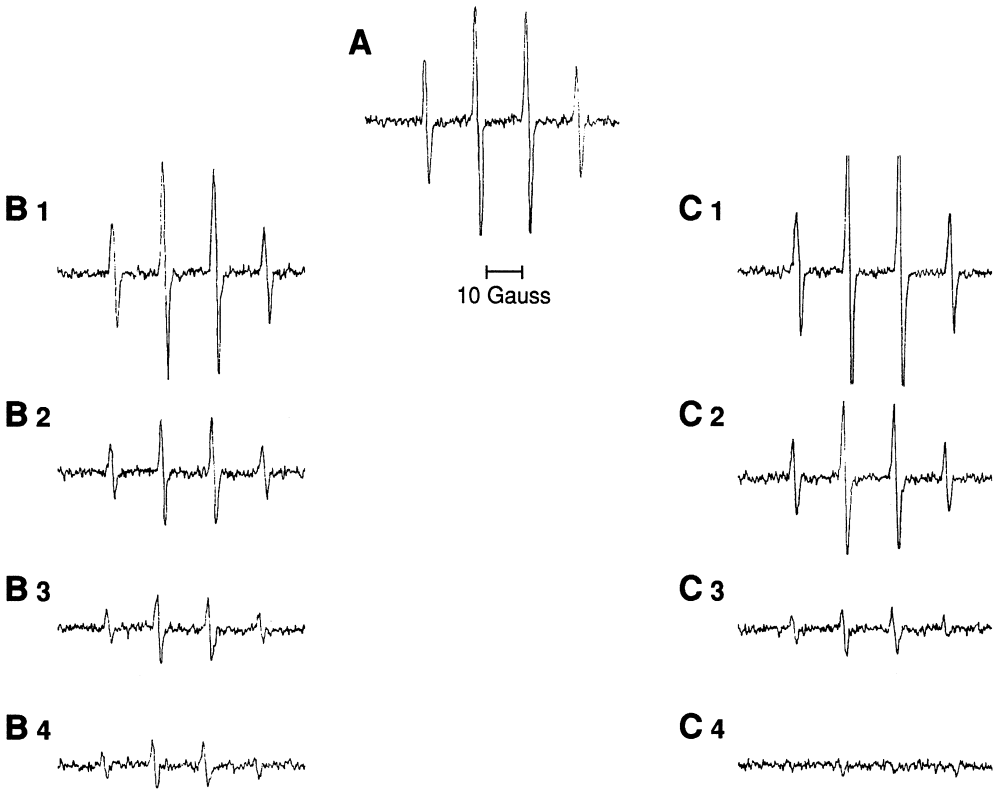

Fig. 3. Effects of fluvastatin and pravastatin upon the electron spin resonance (ESR) spectra of DMPO-OH generated by the Fenton reaction. The ESR spectra after the reaction of 1 mM DMPO with 200 μM H_2O_2 and 100 μM $FeCl_2$ in the absence (A) and in the presence of several concentrations of fluvastatin ($B_1 = 0.01$ mM; $B_2 = 0.1$ mM; $B_3 = 0.5$ mM; $B_4 = 1$ mM) or pravastatin ($C_1 = 0.01$ mM; $C_2 = 0.1$ mM; $C_3 = 0.5$ mM; $C_4 = 1$ mM).

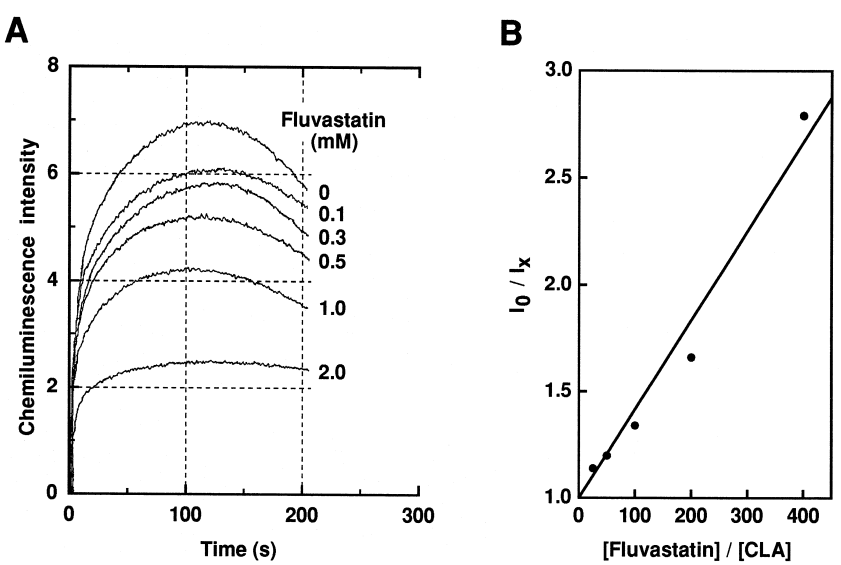

Fig. 4. (A) Chemiluminescence from 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) by its interaction with superoxide and its inhibition by fluvastatin at 25°C. Xanthine oxidase (XOD) was added to the solution of xanthine (X), CLA and 0.08 μM catalase in the presence of several concentrations of fluvastatin at time 0. [X] = 100 μM; [XOD] = 0.016 unit/l; [CLA] = 5.0 μM. (B) Plot of I_o/I_x as a function of [fluvastatin]/[CLA] for the system X-XOD-CLA-fluvastatin. These data are shown in (A).

4. Discussion

The present study has demonstrated that fluvastatin scavenged the active oxygen species and inhibited the formation of thiobarbituric acid reactive substances both in vivo and in vitro in rat liver microsomes.

Ingelman-Sundberg (1986) has reported that cytochrome P-450 links with fp_2, and that superoxide anions (O_2^-) generated by cytochrome P-450 in the interaction with iron in the hydrophobic interior of the membrane are active oxygen species able to initiate lipid peroxidation (Hu et al., 1994; Bestervelt et al., 1995). In contrast, some reports indicate the non-involvement of cytochrome P-450 in NADPH-dependent lipid peroxidation in microsomes (Ernster et al., 1982; Davis et al., 1987). Puntarulo and Cederbaum (1996) have demonstrated that a physiological source of catalytically active iron, such as ferritin, contributes to the increased production of reactive oxygen species, resulting in cellular damage which is dependent upon cytochrome P-450. Thus, cytochrome P-450 is related to the generation of reactive intermediates of partially reduced oxygen, such as O_2^-, hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$), which are formed at the cytochrome P-450 active center as a result of the uncoupling of monooxygenase reactions (Karuzina and Archakov, 1994). In addition, cytochrome P-450 inactivation involves the formation of $\cdot OH$ from H_2O_2 in the Fe^{2+}-catalyzed Fenton reaction or from H_2O_2 and O_2^- in the Fe^{3+}-catalyzed Harber and Weiss (1934) reaction (Winterbourn, 1987; Nakamura, 1990; Karuzina and Archakov, 1994).

In a recent report, fluvastatin selectively and competitively inhibited a major drug metabolizing enzyme (CYP2C9) in vivo and in vitro (Transon et al., 1995, 1996). The lipid-rich microsomal membranes are potential targets of injury in cells exposed to active oxygen species (Afanas'ev et al., 1993). In our in vivo experiments, lipid peroxidation in microsomes significantly decreased 1 h after the administration of fluvastatin, but subsequently did not change until 24 h (Fig. 1), although fluvastatin at any dose used did not affect either cytochrome P-450 or fp_2 in microsomes. This may indicate that at the concentrations of fluvastatin used, the O_2^- generated via cytochrome P-450 and accumulated outside the enzyme's active center was not enough to affect lipid peroxidation. Therefore, these data clearly imply that the inhibition of lipid peroxidation in microsomes by fluvastatin may be due to a direct interaction with membrane phospholipids and that in vivo inhibition of NADPH-dependent lipid peroxidation by fluvastatin may not be related to cytochrome P-450 and/or fp_2 in hepatic microsomes. Therefore we tried to identify possible mechanisms other than cytochrome P-450-mediated lipid peroxidation.

Butylated hydroxytoluene is a phenolic antioxidant that is widely used in the food industry (Thompson et al., 1989). Moreover, probucol is also highly lipophilic, and the antioxidant property of both drugs is partly due to their free radical scavenging effect (Valoti et al., 1989; Hiramatsu et al., 1994). Lovastatin, simvastatin and fluvastatin are well known lipid-soluble HMG-CoA reductase inhibitors (Koga et al., 1990; Satoh et al., 1995). Since the properties of fluvastatin are similar to those of pravastatin except for the tissue-selective inhibition of cholesterol synthesis (Yamamoto et al., 1995), it may be suggested that pravastatin causes in vitro inhibition of NADPH-dependent lipid peroxidation. When the inhibition of active oxygen species generation was chemically observed with the xanthine and Fenton systems, fluvastatin showed scavenging activity for O_2^- and $\cdot OH$, but pravastatin showed no scavenging activity for O_2^- (Figs. 3 and 4). Fluvastatin, which has a 4-fluorophenyl group at the 3 position of the indole, is more highly lipophilic than pravastatin because of the hydrophilicity of the β-hydroxy group at the 6 position of decaline. Therefore, it is likely that fluvastatin operates most effectively in lipid-rich microsomal membranes to suppress the propagation (or initiation) of lipid peroxidation, but pravastatin is less active because of its hydrophilicity. In fact, fluvastatin markedly decreased the dose–response curve for the formation of thiobarbituric acid reactive substances in microsomes (Fig. 2), and the inhibitory potency of the compounds on the formation of thiobarbituric acid reactive substances by in vitro lipid peroxidation decreased in the order of butylated hydroxytoluene > probucol ≥ fluvastatin > pravastatin (Table 3). This may indicate that fluvastatin scavenges the free radicals generated through the $FeCl_2$ system in microsomal membranes, but pravastatin does not quench O_2^- induced by the reaction in microsomes.

Our previous paper (Yamamoto et al., 1995) demonstrated that fluvastatin decreased sterol synthesis in liver which, in turn, caused a decrease in the serum cholesterol level, and that the lowering of the serum cholesterol level is due to an increase in the number of LDL receptors and that the induction of LDL receptors in the liver, by lowering intracellular free cholesterol, is a time-consuming process. However, plasma levels of fluvastatin after the oral administration of the drug are not comparable to those of the in vitro study. Serum LDL is modified by lipid peroxidation initiated by cell-derived free radicals and this peroxidation is accelerated by O_2^- and H_2O_2 (Afanas'ev et al., 1993; Rifici and Khachadurian, 1996). Recently, Hussein et al. (1997) reported that fluvastatin was a potent inhibitor of LDL oxidation in vitro, but its inhibitory effect on LDL oxidation was not related to the antioxidant effect of the drug. However, they used a lower concentration of fluvastatin and did not directly determine the effect of O_2^- and $\cdot OH$ on fluvastatin. Our in vivo results showed that low doses of fluvastatin inhibited the formation of thiobarbituric acid reactive substances in the liver, while high doses of fluvastatin did not change the formation of these substances (Table 2). These results can be explained by the consideration that the time course of inhibition of lipid

peroxidation by fluvastatin may be different after low and high doses and that some inhibitory effects of higher doses may occur at other times. Oxidative modification of LDL is a key event in early atherogenesis (Witztum and Steinberg, 1991), and fluvastatin therapy decreases plasma levels of LDL cholesterol and reduces the susceptibility to oxidation of LDL from hypercholesterolemic patients (Hoffman et al., 1992; Hussein et al., 1997). In our experiments, fluvastatin scavenged O_2^- and ·OH, and inhibited lipid peroxidation in vitro and in vivo. This may be due to the scavenging by fluvastatin of the free radicals generated in rat liver microsomes.

The present study demonstrates that fluvastatin may act as an antioxidant through its free radical scavenging capacity.

References

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.

Bestervelt, L.L., Vaz, A.D.N., Coon, M.J., 1995. Inactivation of ethanol-inducible cytochrome P-450 and other microsomal P-450 isozymes by *trans*-4-hydroxy-2-nonenal, a major product of membrane lipid peroxidation. Proc. Natl. Acad. Sci. USA 92, 3764–3768.

Davis, H.W., Suzuki, T., Schenkman, J.B., 1987. Oxidation of esterified arachidonate by rat liver microsomes. Arch. Biochem. Biophys. 252, 218–228.

Ernster, L., Nordenbrand, K., Orrenius, S., 1982. Microsomal lipid peroxidation: mechanism and some biomedical implications, In: Yagi, K. (Eds.), Lipid Peroxides in Biology and Medicine. Academic Press, New York and London, pp. 55–79.

Finkelstein, E., Rosen, G.M., Rauckman, E.J., 1980. Spin trapping of superoxide and hydroxyl radical: practical aspects. Arch. Biochem. Biophys. 200, 1–16.

Gotoh, N., Niki, E., 1992. Rates of interactions of superoxide with vitamin E, vitamin C and related compounds as measured by chemiluminescence. Biochim. Biophys. Acta 1115, 201–207.

Gotoh, N., Niki, E., 1994. Measurement of superoxide reaction by chemiluminescence. Methods in Enzymology 233, 154–160.

Harber, F., Weiss, J., 1934. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. R. Soc. London. Ser. A 147, 332–351.

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.

Hoshi, K., Senda, N., Fujino, S., 1989. Acute effect of amitriptyline, phenobarbital or cobaltous chloride on δ-aminolevulinic acid synthetase, heme oxygenase and microsomal heme content and drug metabolism in rat liver. Japan J. Pharmacol. 50, 289–293.

Hu, Y., Mishin, V., Johansson, I., Bahr, C., Cross, A., Ronis, M.J.J., Badger, T.M., Ingelman-Sundberg, M., 1994. Chlormethiazole as an efficient inhibitor of cytochrome P450 2E1 expression in rat liver. J. Pharmacol. Exp. Ther. 269, 1286–1291.

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.

Ingelman-Sundberg, M., 1986. Cytochrome P-450 organization and membrane interactions. In: Montellano, P.R.O. (Ed.), Cytochrome P-450. Plenum Press, New York, pp. 119–160.

Jordan, R.A., Schenkman, J.B., 1982. Relationship between malondialdehyde production and arachidonate consumption during NADPH-supported microsomal lipid peroxidation. Biochem. Pharmacol. 31, 1393–1400.

Karuzina, I.I., Archakov, A.I., 1994. Hydrogen peroxide-mediated inactivation of microsomal cytochrome P450 during monooxygenase reactions. Free Radic. Biol. Med. 17, 557–567.

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.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Nakamura, M., 1990. Lactoferrin-mediated formation of oxygen radicals by NADPH-cytochrome P-450 reductase system. J. Biochem. 107, 395–399.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Omura, T., Sato, R., 1964. The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem. 239, 2370–2378.

Ottolenghi, A., 1959. Interaction of ascorbic acid and mitochondrial lipides. Arch. Biochem. Biophys. 79, 355–363.

Phillips, A.H., Langdon, R.G., 1962. Hepatic triphosphopyridine nucleotide-cytochrome c reductase: isolation, characterization, and kinetic studies. J. Biol. Chem. 237, 2652–2661.

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.

Rifici, V.A., Khachadurian, A.K., 1996. Oxidation of high density lipoproteins: characterization and effects on cholesterol efflux from J774 macrophages. Biochim. Biophys. Acta 1299, 87–94.

Rudling, M., Collins, V.P., 1996. Low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels are coordinately reduced in human renal cell carcinoma. Biochim. Biophys. Acta 1299, 75–79.

Satoh, K., Yamamoto, A., Nakai, T., Hoshi, K., Ichihara, K., 1995. Effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on mitochondrial respiration in ischaemic dog hearts. Br. J. Pharmacol. 116, 1894–1898.

Thompson, D.C., Cha, Y., Trush, M.A., 1989. The peroxidase-dependent activation of butylated hydroxyanisole and butylated hydroxytoluene (BHT) to reactive intermediates. J. Biol. Chem. 264, 3957–3965.

Transon, C., Leemann, T., Vogt, N., Dayer, P., 1995. In vivo inhibition profile of cytochrome $P450_{TB}$ (CYP2C9) by (±)-fluvastatin. Clin. Pharmacol. Ther. 58, 412–417.

Transon, C., Leemann, T., Dayer, P., 1996. In vitro comparative inhibition profiles of major human drug metabolising cytochrome P450 isozymes (CYP2C9, CYP2D6 and CYP3A4) by HMG-CoA reductase inhibitors. Eur. J. Clin. Pharmacol. 50, 209–215.

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.

Winterbourn, C.C., 1987. The ability of scavengers to distinguish ·OH production in the iron-catalyzed Haber–Weiss reaction: Comparison of four assays of ·OH. Free Radic. Biol. Med. 3, 33–39.

Witztum, J.L., Steinberg, D., 1991. Role of oxidized low density lipoprotein in atherogenesis. J. Clin. Invest. 88, 1785–1792.

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.