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Protective Effects of Fluvastatin Against Reactive Oxygen Species Induced DNA Damage and Mutagenesis

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Oxidative stress may be an important factor in the development of diabetic complications. Advanced glycation end-products have drown attention as potential sources of oxidative stress in diabetes. We investigated the protective effects of fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, on oxidative DNA damage from reactive oxygen species or advanced glycation end-products *in vitro*, as well as effects of main fluvastatin metabolites and other inhibitors of the same enzyme, pravastatin and simvastatin. Protective effects were assessed in terms of the DNA breakage rate in a single-stranded phage DNA system *in vitro*. DNA was exposed to either reactive oxygen species or advanced glycation end-products. Fluvastatin and its metabolites showed a strong protective effect comparable to those seen with thiourea and mannitol, though pravastatin and simvastatin did not exert clear protective effects. Furthermore, fluvastatin reduced the mutagenesis by reactive oxygen species or advanced glycation end-products in *Salmonella typhimurium* test strains. Both pravastatin and simvastatin still lacked protective activity. Fluvastatin and its metabolites protect against oxidative DNA damage and may reduce risk of consequent diabetic complications.

Keywords: fluvastatin, reactive oxygen species, oxidative DNA damage, advanced glycation end-products, *Salmonella typhimurium* TA102 and TA104, mutagenicity

INTRODUCTION

Fluvastatin (FV), or (±)-(3RS,5RS,6E)-sodium-7-[3-(4-fluorophenyl)-1-(1-methyl-ethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoate (Figure 1), is a new 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor used as a hypolipidemic drug[1].

A previous study has indicated that FV has hydroxyl radical ($HO^{\cdot}$) and superoxide radical ($O_2^{\cdot -}$) scavenging activity *in vitro*[2,3], and FV was found to reduce the level of serum thiobarbituric acid-reactive substances (TBARS) in cholesterol-fed rabbits[4,5].

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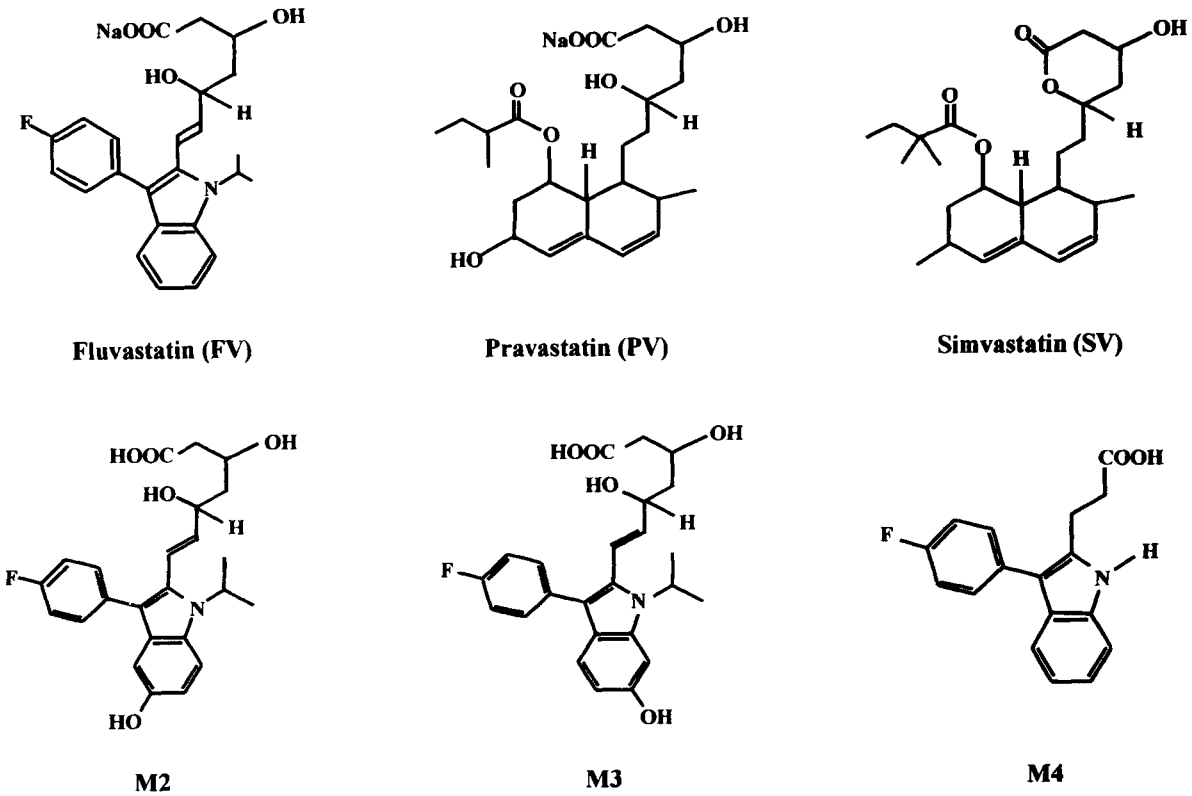

FIGURE 1 Structures of fluvastatin and its major metabolites (M2, M3, and M4), pravastatin, and simvastatin

TABLE I Test materials used in this study

Test material	*Abbreviation or formula*
Fluvastatin	FV
Fluvastatin metabolites	M2, M3, M4
Pravastatin	PV
Simvastatin	SV
Hydroxyl radical	$HO^{\cdot}$
Superoxide radical	$O_2^{\cdot-}$
Hydrogen peroxide	H_2O_2
t-butylhydroperoxide	*t*-BuOOH
Xanthine	X
Xanthine oxidase	XO
Adriamycin	ADR
Ascorbic acid	AsA
Advanced glycation end-products	AGE
Advanced glycation end-products with Bovine serum albumin	AGE-BSA

An increased plasma cholesterol concentration in hypercholesterolemic patients is a major risk factor for atherosclerosis. Impaired removal of plasma low density lipoprotein (LDL) in these patients results in abnormal persistence of LDL in the plasma, allowing more time for oxidative modification to occur[4]. Oxidation of LDL has been linked to atherogenesis[6,7]. According to this hypothesis, oxidized LDL are recognized and taken up by macrophages via the scavenger receptor pathway. FV shows protective effects against oxidation of LDL *in vitro*[4]. Thus FV may help reduce the risk of atherosclerosis by protecting LDL from oxidative modification as well as reducing plasma LDL levels[8]. Reactive oxygen species (ROS), including free radicals such as $HO^{\cdot}$ and $O_2^{\cdot-}$, and molecules such as hydrogen peroxide, are thought to play a major role in the

etiology of a wide variety of diseases including cancer and diabetes, as well as in aging[9].

Increased oxidative stress in diabetes has been implicated in the pathogenesis of diabetic complications[10]. Both an increase in ROS and a decrease in the antioxidant defense mechanism lead to an increase in oxidative stress in diabetes[10]. Advanced glycation end-products (AGE) are known to produce ROS mainly through the glycation[11]. During the process of both glucose autoxidation and glycation, including the formation of AGE, $O_2^{\cdot-}$, hydrogen peroxide, and $HO^{\cdot}$ may be formed[10]. Oxidative stress may be an important pathogenetic factor in the development of diabetic vascular complications [12].

ROS as well known to cause DNA damage and induce mutation, while antioxidative activity inhibits mutagenicity [13]. Mechanisms of ROS-induced cellular injury, including membrane lipid peroxidation, oxidation of structural and functional proteins and amino acids, nucleic acid base damage, DNA strand breaks, and DNA strand cross-linkage, may result in reversible or irreversible consequences including cellular repair, proliferation, differentiation, transformation, and apoptosis or cell death [14]. Antioxidants play an important role in protecting the cell against damage caused by ROS, and also may inhibit mutagenicity induced by ROS.

In the present study we investigated the protective effects of FV on ROS- and AGE-caused DNA damage using a single stranded-DNA phage (øX174RFI), and antimutagenicity effects of FV on ROS- and ADR-induced mutagenesis in *Salmonella typhimurium* TA102 and TA104 test strains *in vitro*. Protective effects of the main metabolites of FV in humans also were investigated. These metabolites are designated M2, representing (±)-(3RS, 5RS, 6E)-7-[3-(4-fluorophenyl)-5-hydroxy-1-(1-methyl-ethyl)-1H-indol-2-yl]-3,5-dihydroxy-5-heptenoic acid; M3, (±)-(3RS,5RS,6E)-7-[3-(4-fluorophenyl)-6-hydroxy-1-(1-methyl-ethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid; and M4, 3-[3-(4-fluorophenyl)-1H-indol-2-yl] propionic acid [1] (Figure 1). In addition, we compared the protective effects of FV on oxidative DNA damage with those of two other HMG-CoA reductase inhibitors, pravastatin (PV) and simvastatin (SV) (Figure 1).

MATERIALS AND METHODS

Reagents

Fluvastatin (FV) was donated from the department of pharmacology, Novartis Pharma (Ibaraki, Japan). FV metabolites (M2, M3, and M4) were synthesized by Tanabe Seiyaku Co. (Osaka, Japan) (Figure 1). Mannitol, *t*-butylhydroperoxide (*t*-BuOOH), xanthine (X), hydrogen peroxide (H_2O_2), and glucose were purchased from Katayama Chemical Industries (Osaka, Japan). Thiourea, Fe^{2+}, ascorbic acid (AsA), Phage øX174RFIDNA (supercoiled, a double-stranded circular form), and xanthine oxidase (XO) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Bovine serum albumin (BSA) was purchased from Sigma Chemical (St-Louis, MO). Adriamycin (ADR) was purchased from Kyowa Hakko Kogyo Co. (Tokyo, Japan) (Table I).

Preparation of Advanced Glycation End-Products Bound with BSA (AGE-BSA)

BSA (16 g) and glucose (30 g) were dissolved in 100 ml phosphate-buffered saline (pH, 7.3) and incubated for 1 month at 37°C. The resulting solution was dialyzed against H_2O and lyophilized to obtain AGE-BSA [15].

Effects on DNA Strand Breakage Induced by ROS in vitro

Protective effects against oxidative DNA damage induced directly by ROS were measured as described previously [16,17]. Each reagent was

dissolved in distilled water. FV was diluted to adjust the final concentrations to 1, 10, 100, and 1000 μM in the reaction mixture. Thiourea and mannitol were used as HO· scavengers. Each test was performed twice or more as its reliability, and obtained satisfactory results between repeated tests *in vitro*.

Fenton reaction

The reaction mixture contained 0.01 mM Fe^{2+}, 0.03% H_2O_2, various concentrations of FV, PV, or SV, or 50 mM of scavengers (thiourea or mannitol), and 0.1 mg/ml DNA in 50 mM sodium cacodylate buffer (pH, 7.5)[16,17]. The mixture was incubated at 37°C for 15 min, and 5 μL of loading buffer was added [40 mM Tris-HCl, pH 8.0; 5 mM EDTA, 40% glycerol, 0.4% sodium dodecyl sulfate, and 0.3% bromphenol blue]. The mixture was applied to 1.2% agarose gels (120 mm × 130 mm × 9.6 mm) containing ethidium bromide (1 μg/ml). Electrophoresis was performed at 100 V for 2 hr, from the negative charge to positive charge at room temperature. Breakage products were visualized by long-wave ultraviolet light. Change into the RFII form (a relaxed circular form) indicated single-strand cuts. The resulting images were photographed and relevant areas were quantified with NIH Image software. DNA damage was expressed as the ratio between the areas representing RFII and RFI (RFII / RFI). RFII / RFI was compared with that in a positive control.

X/XO system

The reaction mixture contained 0.2 mM X, 0.03 U of XO, various concentrations of FV, PV, or SV, or 1000 μM scavenger (thiourea or mannitol), and 200 ng of DNA per electrophoretic lane in 50 mM sodium cacodylate buffer (pH, 7.5) [16,17]. The mixture was incubated at 37°C for 15 min and electrophoresed in agarose gels as described above.

T-BuOOH

The reaction mixture contained 100 mM *t*-BuOOH, various concentrations of FV, PV, or SV, or 1000 μM scavenger (thiourea or mannitol), and 200 ng DNA per electrophoretic lane in 50 mM sodium cacodylate buffer (pH, 7.5) [16,17]. The mixture was incubated at room temperature for 7 days and electrophoresed in agarose gels as described above.

ADR

The reaction mixture contained 0.8 mM ADR, various concentrations of FV, PV, or SV, or 50 mM scavenger (thiourea or mannitol), and 200 ng DNA per electrophoretic lane in 50 mM sodium cacodylate buffer (pH, 7.5) [16,17]. The mixture was incubated at 37°C for 30 min and electrophoresed in agarose gels as described above.

AGE-BSA

The reaction mixture contained AGE-BSA (20 μg/ml), various concentrations of FV, PV, or SV, or 50 mM scavenger (thiourea or mannitol), and 200 ng DNA per electrophoretic lane in 50 mM sodium cacodylate buffer (pH, 7.5) [16,17]. The mixture was incubated at room temperature for 7 days and electrophoresed in agarose gels as described above.

Effects of FV on the Mutagenicity of ROS in *Salmonella Typhimurium* TA102 and TA104 Strains

The effects of antioxidants on the mutagenicity of ROS were examined using a method previously described [18]. *S. typhimurium* TA102 and TA104 were used as the test strains, obtained by the courtesy of Dr. B.N. Ames, Division of Biochemistry and Molecular Biology, University of California Berkeley, CA. These strains were inoculated into Oxoid nutrient broth No. 2, supplemented with 25 μg/ml of ampicillin (for TA102 and TA104) and 2 μg/ml of tetracycline (for

TA102), and cultured for about 12 hr at 37°C with agitation in a water bath. The mutation assay was carried out according to Ames *et al.*[19] with some modification. FV was diluted to result in final concentrations of 0.5, 0.75, and 1.0 μmol/plate. ADR, H_2O_2, *t*-BuOOH, X/XO, and AGE-BSA each were used as ROS generating systems, while AsA, thiourea, and mannitol were tested as antioxidants. Each reagent was dissolved in distilled water.

TABLE II DNA strand breakage induced by various reactive oxygen species-generators: Inhibition by FV, its metabolites, related compounds, and antioxidants.

Additions	*Conc.(μM)*	*DNA damage from ROS-generating system*				
		Fenton reaction	*X / XO*	*t-BuOOH*	*ADR*	*AGE-BSA*
None		3.52	4.99	0.79	0.24	5.05
+ FV	1	2.82	-	-	0.30	-
	10	2.46	-	-	0.30	3.84
	100	1.71	4.20	0.39	0.25	3.13
	1000	0.73	2.21	0.35	0.04	0.91
+ M2	1	1.67	-	-	0.27	3.58
	10	0.93	-	-	0.21	4.40
	100	-	3.59	0.55	-	-
	1000	-	1.97	0.12	-	-
+ M3	0.3	1.56	-	-	-	-
	1	-	-	-	0.22	4.21
	3	1.30	-	-	-	-
	10	-	-	-	0.23	4.83
	100	-	2.77	0.51	-	-
	1000	-	0.72	0.08	-	-
+ M4	1	2.75	-	-	-	4.39
	10	2.15	-	-	-	4.14
	100	1.40	7.44	0.49	0.25	3.41
	1000	0.55	7.49	0.50	0.20	1.18
+ PV	10	3.10	-	-	-	5.66
	100	3.17	4.19	0.57	0.28	5.96
	1000	2.82	2.33	0.61	0.27	5.33
+ SV	10	3.41	-	-	-	5.46
	100	3.81	13.17	1.00	0.28	5.28
	1000	3.98	16.97	4.98	0.27	5.01
+ thiourea	1000	-	4.09	1660	-	-
	50000	1.04	-	-	0.02	0.17
+ mannitol	1000	-	3.61	0.67	-	-
	50000	1.05	-	-	0.11	0.29

DNA damage is expressed as the ratio between the areas representing RF II DNA and RF I DNA (RF II / RF I) on electrophoretic gels. M2, M3, and M4 designate principal FV metabolites in humans. FV, fluvastatin; PV, pravastatin; SV, simvastatin; ROS, reactive oxygen species; X, xanthine; XO, xanthine oxidase; *t*-BuOOH, t-butylhydroperoxide; ADR, Adriamycin; AGE-BSA, advanced glycation end products with bovine serum albumin. -, not examined.

A 0.1-ml volume of various concentrations of FV or antioxidant was placed in a small test tube, to which 0.1 ml of ROS generation solution was added. Then, 0.5 ml of 0.1 M sodium phosphate-buffered solution (pH, 7.4) and 0.1 ml of the test strain suspension was placed into the test tube. The mixture was incubated for 20 min at 37°C with agitation in a water bath. After being mixed with 2 ml of agar kept at 45°C, the suspension was poured quickly onto minimal glucose agar plates. The plates were incubated for about 48 hrs at 37°C, and the number of His^+ revertant colonies developed was counted. Duplicate plates were prepared and observed for each dose. The protocol was performed twice or more as its reliability, and obtained satisfactory results between repeated tests *in vitro*.

RESULTS

Effects on DNA Strand Breakage Induced by ROS *in vitro*

Results of electrophoresis indicating breakage of phage DNA strands in the Fenton reaction are presented quantitatively in the Table II and shown in Figure 2A. When DNA alone was electrophoresed, no DNA strand breakage (RFII) was noted (lane 1). Upon addition of Fe^{2+} and H_2O_2, most of the DNA strands (RFI) were broken into RFII form (lane 2). FV and its metabolites inhibited Fenton reaction-induced DNA strand breakage in a dose-dependent manner, representing a protective effect against DNA strand breakage. FV at 1 mM (lane 5) significantly inhibited DNA strand breakage, resulting in an RFII / RFI ratio for FV equivalent to the ratio attainable with 50 mM thiourea (lane 3) or mannitol (lane 4). In addition, M2 and M3 provided even stronger protection against DNA strand breakage than FV.

With X/XO as the ROS generator, FV and its metabolites M2 and M3 again showed protection against DNA strand breakage (Table II). M3 at 1 mM was more effective than FV at the same concentration. The protective effects of FV against *t*-BuOOH-induced DNA strand breakage were stronger than those of thiourea and mannitol (Table II). The metabolites of FV also exhibited protective effects against *t*-BuOOH-induced DNA strand breakage; M2 and M3 showed significantly stronger protection against breakage than FV. FV at 1 mM exhibited protective effects against ADR-induced DNA strand breakage, though the metabolites of FV did not (Table II).

Protective effects of FV against DNA strand breakage were compared with those of PV or SV (Table II; Figure 2A). FV exhibited protective effects against Fenton reaction-, X/XO-, *t*-BuOOH-, and ADR-induced breakage. PV at 1 mM showed protective activity against Fenton reaction-, X/XO-, and *t*-BuOOH-induced DNA strand breakage, but SV did not.

Effects of FV on DNA Strand Breakage Induced by AGE-BSA *in vitro*

Results of electrophoresis indicating the breakage of phage DNA strands induced by AGE-BSA are shown in the Table II and Figure 2B. When AGE-BSA was added to the incubation mixture, the DNA strands were broken (lane 2). Addition of FV at 1 mM (lane 5), resulted in protective effects on AGE-BSA-induced DNA strand breakage. M2, M3, and M4, metabolites of FV, also exhibited protective effects against AGE-BSA-induced breakage. On the other hand, no protective effects of PV and SV were shown in this system, and most of the DNA strands had been broken into the RFII form.

Effects of FV on Mutagenicity of ROS in *S. Typhimurium* TA102 and TA104 Strains

The effects of FV on the mutagenicity of ADR were examined using the Ames test with *S. typhimurium* TA102 and TA104 test strains (Figure 3).

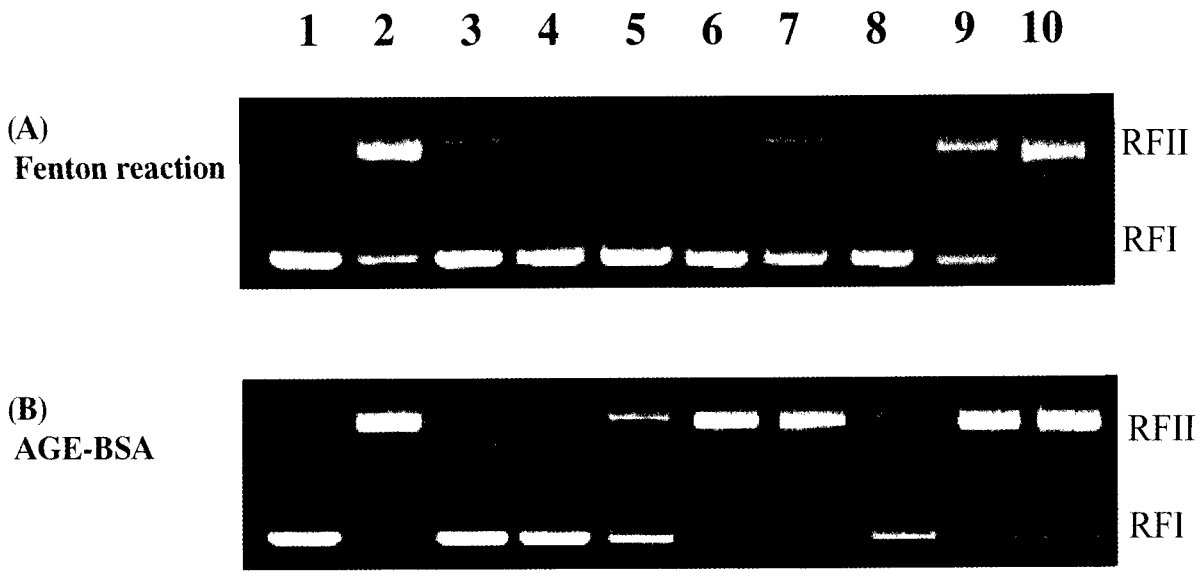

FIGURE 2 DNA strand breakage induced by reactive oxygen species-generating systems and inhibition by FV, metabolites (M2, M3, and M4), related compounds, and antioxidants. Phage øX174RFI DNA was incubated with ROS-generating reagents and the test substance in 50 mM sodium cacodylate buffer (pH, 7.5) prior to electrophoresis in agarose gel. The direction of electrophoresis was from top to bottom. Change into the RF II form (relaxed circular form) indicates single-strand cuts. A, DNA strand breakage induced by the Fenton reaction. Lane 1, DNA only; 2, $Fe^{2+} + H_2O_2$; 3, $Fe^{2+} + H_2O_2$ + thiourea; 4, $Fe^{2+} + H_2O_2$ + mannitol; 5, $Fe^{2+} + H_2O_2$ + 1 mM FV; 6, $Fe^{2+} + H_2O_2$ + 10 μM M2; 7, $Fe^{2+} + H_2O_2$ + 3 μM M3; 8, $Fe^{2+} + H_2O_2$ + 1 mM M4; 9, $Fe^{2+} + H_2O_2$ + 1 mM PV; and 10, $Fe^{2+} + H_2O_2$ + 1 mM SV. B, DNA strand breakage induced by AGE-BSA. Lane 1, DNA only; 2, AGE-BSA; 3, AGE-BSA + thiourea; 4, AGE-BSA + mannitol; 5, AGE-BSA + 1 mM FV; 6, AGE-BSA + 10 μM M2; 7, AGE-BSA + 10 μM M3; 8, AGE-BSA + 1 mM M4; 9, AGE-BSA + 1 mM PV; and 10, AGE-BSA + 1 mM SV. M2, M3, and M4 designate principal FV metabolites. FV, fluvastatin; PV, pravastatin; SV simvastatin; ROS, reactive oxygen species; AGE-BSA, advanced glycation end products with bovine serum albumin

The number of ADR-induced revertant colonies of TA102 was reduced to about 60% and 30% respectively by adding to 0.5 or 1.0 μmol/plate of FV. AsA reduced ADR-induced mutagenic potency to a similar extent. FV reduced ADR-induced mutagenic potency on the TA104 test strain as well, but without dose-dependency.

FV inhibited *t*-BuOOH-induced reversion in TA104, but did not affect the number of revertant colonies of TA102 (Figure 4). In TA104, FV reduced *t*-BuOOH-induced mutagenic potency similarly to AsA.

FV reduced the number of H_2O_2-induced revertant colonies of TA102 and TA104; results were comparable to those with thiourea (Figure 5). Though mannitol was tested as an $HO^{\cdot}$ scavenger, it did not reduce the H_2O_2-induced mutagenic potency in this study.

The effects of FV on the mutagenicity of the X/XO system also were examined using the Ames test with *S. typhimurium* TA104 test strains (Figure 6). The number of revertant colonies was reduced to about 70% by adding 1.0 μmol of FV to culture plates.

Effects of FV on Mutagenicity of AGE-BSA in *S. Typhimurium* TA104 Strain

The effects of FV on the mutagenicity of AGE-BSA were examined by the Ames test using the TA104 *S. typhimurium* test strain (Figure 7). With only AGE-BSA added, the

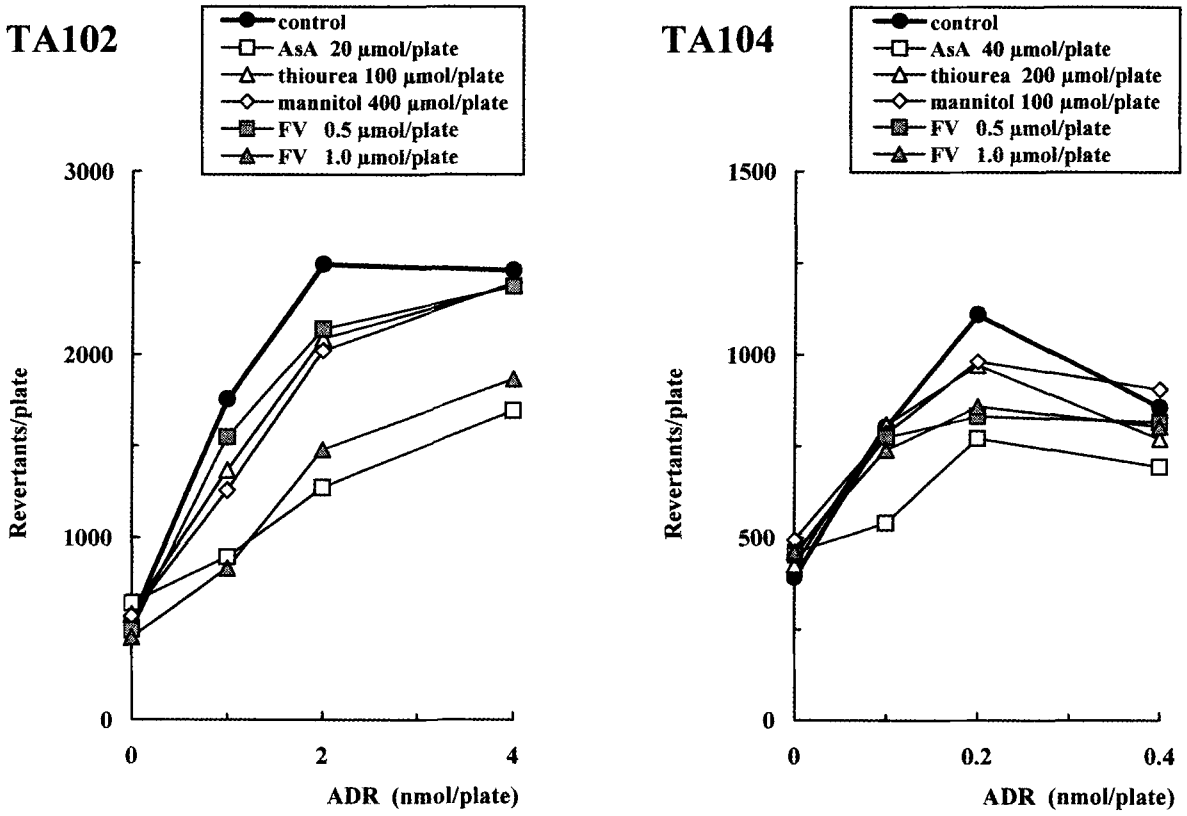

FIGURE 3 Effects of FV on mutations induced by ADR in *Salmonella typhimurium* TA102 and TA104 test strains. FV, fluvastatin; ADR, Adriamycin; AsA, ascorbic acid

number of revertant colonies of TA104 treated with AGE-BSA increased dose-dependently. On the other hand, the number of revertant colonies was reduced to about 70% and 50%, by adding 0.5 and 1.0 µmol of FV per plate, respectively, showing a dose-dependent suppressive activity. FV reduced AGE-BSA-induced mutagenic potency, with no significant difference evident between FV and AsA.

DISCUSSION

Ames [14] has shown that oxygen-derived free radicals may play a major role as endogenous initiators and promoters of DNA damage and mutation that may be related to cancer, diabetes, heart disease, and other age-related diseases. Antioxidants may represent an important defense against these agents, and many antioxidants are being studied as anticarcinogens. FV, developed as an HMG-CoA reductase inhibitor, shows protective effects against oxidation of LDL [4,5,7]. In the present study we investigated the protective effects of FV and its metabolites in humans (M2, M3, and M4) on oxidative DNA damage induced by ROS *in vitro*. In addition, we compared the protective effects of FV against oxidative DNA damage with those of two other HMG-CoA reductase inhibitors, PV and SV.

Using a phage DNA single-strand breakage system *in vitro*, we examined the suppressive

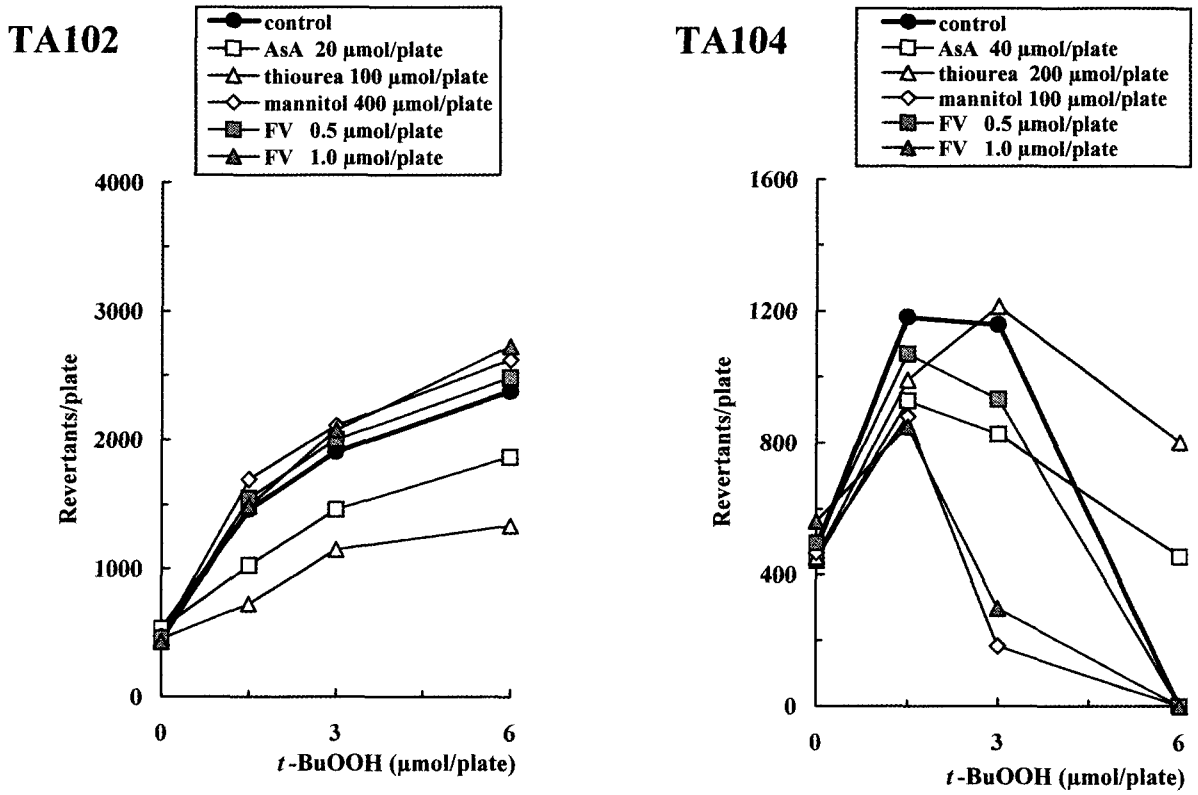

FIGURE 4 Effects of FV on mutations induced by *t*-BuOOH in *Salmonella typhimurium* TA102 and TA104 test strains. FV, fluvastatin; *t*-BuOOH, *t*-butylhydroperoxide; AsA, ascorbic acid

activity of FV on oxidative DNA damage directly induced by the Fenton reaction, the X/XO system, *t*-BuOOH, and ADR. FV exhibited protective effects against phage DNA single-strand breakage induced by these results, in the order of Fenton reaction > *t*-BuOOH > X/XO system > ADR. FV reduced DNA strand breakage induced by the Fenton reaction to the same extent as thiourea and mannitol, well-known $HO^{\cdot}$ scavengers. On the other hand, FV was more effective in reducing X/XO- or *t*-BuOOH-induced DNA single strand breakage than either thiourea or mannitol. These results indicated that FV may have the ability to act not only as an HMG-CoA reductase inhibitor, but also an ROS scavenger. In comparison with other HMG-CoA reductase inhibitors PV and SV, only FV showed consistently effective activity for the inhibition of breakage of phage DNA strands. The antioxidative effects of FV, then, may derive from its unique chemical structure while includes a double bond conjugated with the fluorophenyl indole moiety[20]. Antioxidative effects of FV are likely to be related to this conjugated double bound[20]. The relative protective effect of M2 or M3, major metabolites of FV, against DNA strand breakage induced by the Fenton reaction was significantly greater than that of FV, on the order of M3 > M2 > FV = M4.

These results suggested that FV might reduce cytotoxicity caused by ROS-induced DNA dam-

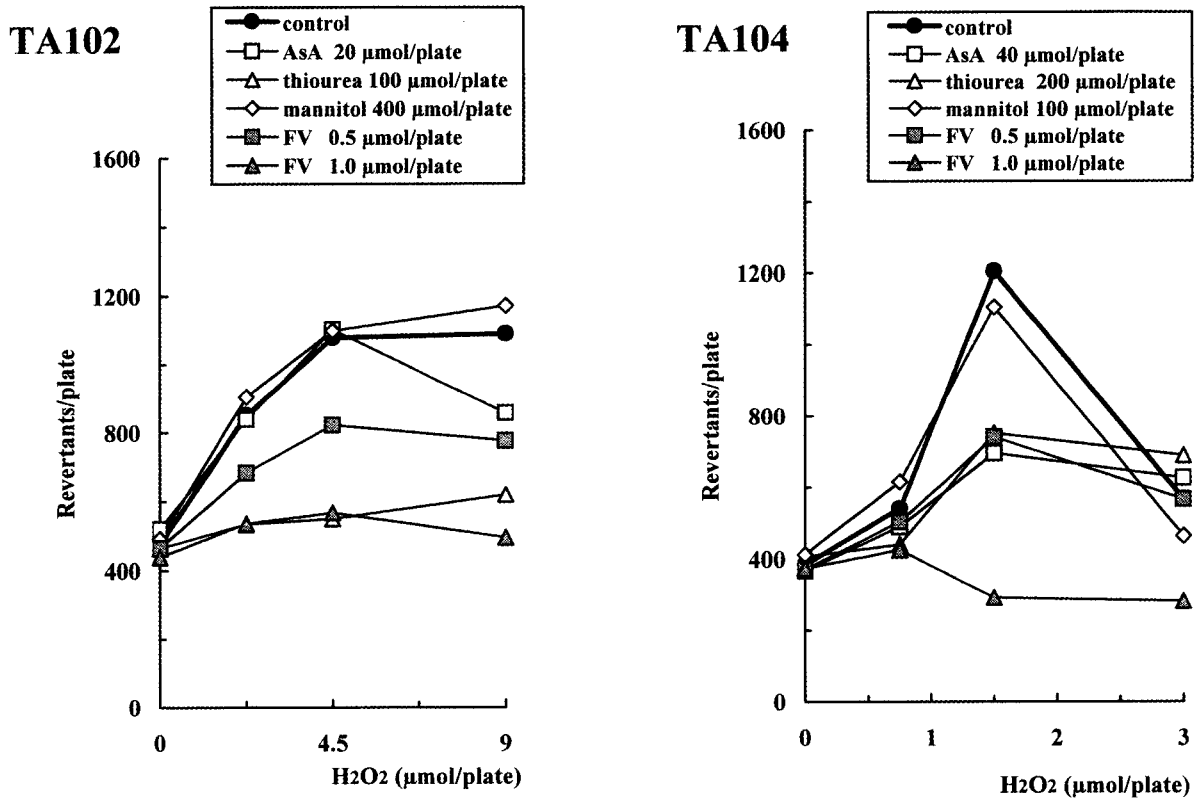

FIGURE 5 Effects of FV on mutations induced by H_2O_2 in *Salmonella typhimurium* TA102 and TA104 test strains. FV, fluvastatin; AsA, ascorbic acid

age, and so we examined the preventive action of FV on the mutagenicity of ROS in the Ames test with *S. typhimurium*. TA102 and TA104 strains were used since they are reported to be more sensitive to oxidative stress than other strains in testing for mutagenesis resulting from DNA damage from generation of free radicals[21,22]. FV inhibited ADR-induced reversion in TA102 and TA104 strains, and no significant difference was seen between FV and AsA. FV caused a reduction of *t*-BuOOH-induced reversion in TA104, and the antimutagenic potency of FV was equal to that of AsA and mannitol. FV reduced H_2O_2-induced mutagenesis in TA102 and TA104 test strains to about the extent that thiourea did, and FV inhibited X/XO-induced reversion in TA104. FV, then, inhibited the mutagenicity induced by several ROS-generating systems, and reduced oxidative DNA damage.

AGE are formed in biologic systems when glucose and other reducing sugars react by nonenzymatic glycation with amino groups of proteins. Long-lived proteins such as collagen and crystalline lens proteins are known to be susceptible to AGE modification, which may play a major role in the complications of diabetes and in age-related diseases [23,24]. AGE increases production of ROS (e.g., $HO^{\cdot}$, $O_2^{\cdot -}$, and hydroperoxy radicals, or metal-oxygen complexes) and has been identified a as potential

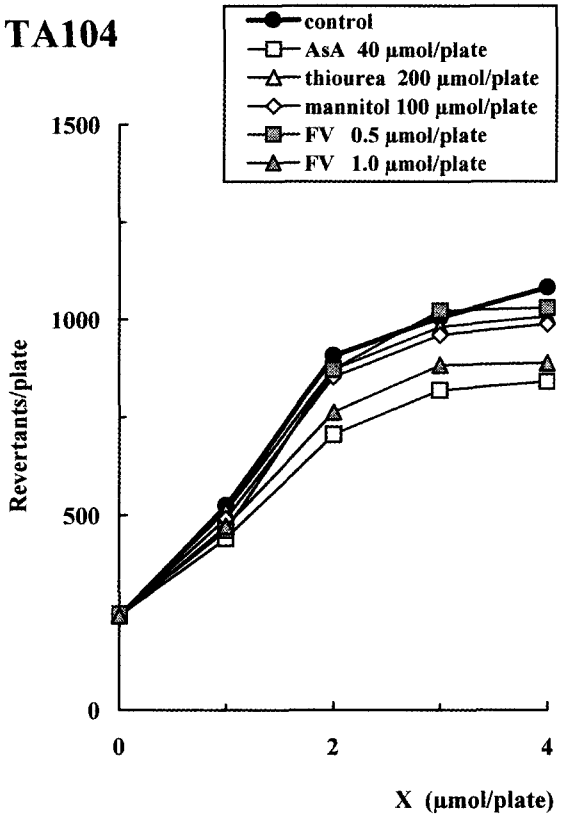

FIGURE 6 Effects of FV on mutations induced by an X/XO system in *Salmonella typhimurium* TA104 test strains. FV, fluvastatin; X, xanthine; XO (1U/ml), xanthine oxidase; AsA, ascorbic acid

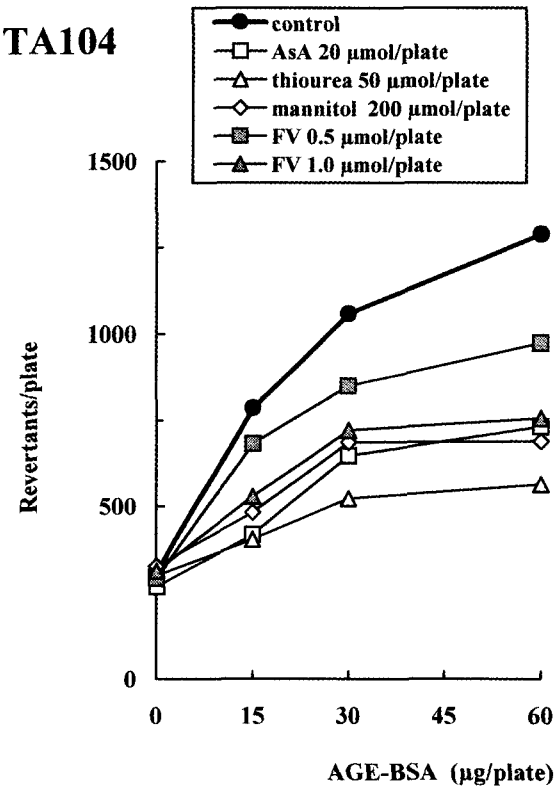

FIGURE 7 Effects of FV on mutations induced by AGE-BSA in *Salmonella typhimurium* TA104 test strains. FV, fluvastatin; AGE-BSA, advanced glycation end products with bovine serum albumin; AsA, ascorbic acid

source of oxidative stress in diabetes [25,26]. Free radicals generated by glycated protein can nearly double the degree of peroxidation of membranes of linoleic/arachidonic acid vesicles. Increased glycation of proteins in diabetes may accelerate vascular wall lipid oxidative modification [27]. Oxidative stress and AGE, then, are closely related processes likely to contribute to diabetic complications [28,29]. In this study, FV and its metabolites exhibited protective effects against phage DNA single-strand breakage induced by AGE-BSA. FV also reduced AGE-BSA-induced mutagenesis in the Ames test.

FV and, to an even greater effect, its metabolites were shown to be far more effective than two other HMG-CoA reductase inhibitors in inhibition of ROS-induced DNA strand breakage and mutagenicity. FV, then, may have clinical value in preventing diabetic complications resulting from oxidative stress.

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