Effects of a Novel Antihyperlipidemic Agent, S-2E, on the Blood Lipid Abnormalities in Homozygous WHHL Rabbits

K. Ohmori, H. Yamada, A. Yasuda, A. Yamamoto, N. Matsuura, and M. Kiniwa

To improve mixed hyperlipidemia in the low-density lipoprotein (LDL) receptor–deficient state, suppression of very-low-density lipoprotein (VLDL) particle production may be an important approach. We previously reported that S-2E, (+)-(S)-*p*-[1-(*p*-*tert*-butylphenyl)-2-oxo-4-pyrrolidinyl] methoxybenzoic acid, suppressed VLDL particle production by inhibiting the biosynthesis of both sterol and fatty acids in the liver. We therefore examined whether S-2E lowered the blood cholesterol and triglyceride (TG) levels simultaneously in homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits, which correspond to human familial hypercholesterolemia. S-2E given orally at doses of 30 to 300 mg/kg significantly lowered serum total cholesterol (TC) levels at 1 week as well as TG at 2 weeks, and the lowering of TC and TG levels by S-2E reached a maximum at 3 to 4 weeks. In contrast, oral administration of pravastatin at doses of 10 to 100 mg/kg resulted in a significant suppression of TC levels (100 mg/kg) but not TG levels. Further analysis of the TC content in fractionated serum of control and S-2E–treated animals showed that suppression of TC level by S-2E is attributable to a decrease in the proportions of VLDL, intermediate-density lipoprotein (IDL), and LDL. It is, therefore, reasonable to assume that S-2E may be useful to improve the blood lipid abnormalities in the LDL receptor–deficient state.

IN PATIENTS with mixed hyperlipidemia such as hypertriglyceridemia and homozygous familial hypercholesterolemia, and especially in low-density lipoprotein (LDL) receptor null type cases, therapy using inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) has almost no effects, because their cholesterol-lowering effects are dependent on the elevated LDL receptor function in liver by competitive inhibition of cholesterol biosynthesis.[1-3] To reduce the blood cholesterol and triglyceride (TG) levels in these patients, the supply of lipids or lipoprotein particles into the blood should be suppressed. Therefore, reduction in the production of very-low-density lipoprotein (VLDL) particles in the liver may be an important approach.

Because of this target, microsomal triglyceride transfer protein (MTP) has been considered to play an important role in the assembly of VLDL particles in the liver and chylomicron particles in the intestine.[4,5] When MTP inhibitors such as compound 9 and Implitapide (Bayer, Leverkusen, Germany) suppress the assembly of VLDL particles, secretion of the lipoproteins is reduced.[6,7] Indeed, in homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits, which correspond to human familial hypercholesterolemia,[8,9] its blockage reduced blood cholesterol and TG levels, but caused hepatic accumulation of TG.[6,7] This accumulation may influence hepatic function. Therefore, another target to enable suppression of VLDL particle production is the limiting of synthesis of necessary components such as cholesterol, fatty acids, and TG.[5] To effectively inhibit synthesis of both sterols and fatty acids, both the rate-limiting enzymes of synthesis for sterols and fatty acids, that is, HMG-CoA reductase and acetyl-CoA carboxylase (EC 6.4.1.2), are notable targets. However, at present, there are no antihyperlipidemic drugs that have the capacity to lower blood total cholesterol (TC) and TG levels simultaneously by inhibition of synthesis of sterols and fatty acids. However, S-2E, (+)-(S)-*p*-[1-(*p*-*tert*-butylphenyl)-2-oxo-4-pyrrolidinyl] methoxybenzoic acid (Fig 1) has been reported to inhibit in vitro synthesis of both sterols and fatty acid in rat liver slices.[10] We previously reported that S-2E noncompetitively inhibited both activities of HMG-CoA reductase and acetyl-CoA carboxylase.[11] In that study, S-2E suppressed the secretion of VLDL-cholesterol (VLDL-C) and TG from the liver. Furthermore, it showed that S-2E lowered the blood TC and TG levels by suppression of the new production of VLDL particles.

In the present study, we examined whether an inhibitor of the synthesis of both sterols and fatty acids, S-2E, could lower the blood TC and TG levels in homozygous WHHL rabbits.

MATERIALS AND METHODS

Chemicals

S-2E was synthesized at Taiho Fine Chemical Co (Tokyo, Japan). Pravastatin-Na (PRV), an HMG-CoA reductase inhibitor, was extracted from a commercially available tablet (Mevalotin; Sankyo, Tokyo, Japan). S-2E ground in an agate mortar was suspended in 0.5% hydroxypropylmethylcellulose (HPMC, Code 60SH50; Shin-Etsu Chemical Co, Tokyo, Japan). PRV was weighed and dissolved in HPMC aqueous solution.

Animals

Two-month-old male WHHL rabbits were purchased from Kitayama LABES (Ina, Nagano, Japan). Throughout the experimental period, the WHHL rabbits were given restricted feeding according to their body weight (BW) (100 g: ≤2 kg BW; 110 g: 2 kg < BW ≤ 2.5 kg; 120 g: >2.5 kg BW). Chow (LRC4; Oriental Yeast Co, Tokyo, Japan) contained 7.1 % water, 18.6 % protein, 3.6 % lipid, 14.2 % fiber, 8.1 % ash, and 48.3 % nitrogen-free extracts. Water was supplied ad libitum and the animals were subjected to a light-dark cycle of 12 hours (light period: 6 AM to 6 PM).

Administration of Test Agents

Vehicle and test agents were given orally by gavage to the rabbits between 9 AM and 10:30 AM. The treatment period was set for 4 weeks.

From the Pharmacobioregulation Research Laboratory, Pharmacology Research Laboratory and OTC Products Research Laboratory, Taiho Pharmaceutical Co, Ltd, Hanno, Japan.

Submitted July 28, 2003; accepted November 6, 2003.

Address reprint requests to Koichi Ohmori, Pharmacobioregulation Research Laboratory, Taiho Pharmaceutical Co, Ltd, 1-27, Misugidai, Hanno, Saitama 357-8527, Japan.

0026-0495/04/5305-0002$30.00/0
doi:10.1016/j.metabol.2003.11.024

S-2E

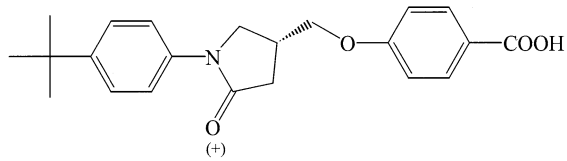

Fig 1. Chemical structure of S-2E: (+)-(S)-*p*- [1-(*p-tert*-butylphenyl)-2-oxo-4-pyrrolidinyl] methoxybenzoic acid. Molecular weight 367.445.

After administering vehicles and test agents, the rabbits were given chow. During the experiments, the rabbits' body weight and food intakes were similar in the control and test agents–treated groups (data not shown).

In Vivo Study in WHHL Rabbits

For homozygous WHHL rabbits, there was a pretreatment period of 4 weeks. During this period, blood was collected from the aural vein once per week between 8 AM and 10 AM before administration of vehicle and test agents. Serum was then obtained by centrifugation. Based on the serum TC and TG values during the pretreatment period, the rabbits were divided into 4 groups (control group, S-2E 30 mg/kg group, S-2E 100 mg/kg group, and S-2E 300 mg/kg group). S-2E was given orally to the rabbits for 4 weeks. During the treatment period, blood was collected from the aural vein once per week, in a manner similar to the measurements and blood sampling conducted during the acclimation period. Serum was then obtained by centrifugation.

The antihyperlipidemic actions of PRV (10, 30, and 100 mg/kg) were evaluated and compared with those of S-2E by the same experimental protocol, using homozygous WHHL rabbits, which were purchased separately.

Measurement of Lipids

TC and TG in the obtained serum were measured by an enzymatic method with an automatic analyzer (7170, Hitachi, Tokyo Japan) (TC: L-CHO S, Wako Pure Chemical Industries, Osaka, Japan; TG: L-TG H, Wako].

Fractionation of Lipoproteins by Ultracentrifugation

Lipoprotein fractionation was performed by ultracentrifugation (Optima TLX Ultracentrifuge; Beckman, Fullerton, CA) employing the method of Hatch and Lees.[12] According to their method, 4 solutions—solution 1 (density = 1.006 g/L), solution 2 (density = 1.045 g/L), solution 3 (density = 1.151 g/L), and solution 4 (density = 1.504 g/L)—were prepared with NaCl and KBr. Then, according to the specific gravity of each, 5 fractions were isolated at 20°C as described below. That is, 490 μL of solution 1 was layered over the surface on 980 μL of serum in a Quick-seal 11 $\times$ 25-mm ultracentrifuge tube (Beckman), using a TLA 120.2 rotor (Beckman). After ultracentrifugation (26,000 $\times$ *g*, 30 minutes), the fraction that floated to the top of the tube was separated as chylomicron. Then, the 980-μL infractant solutions were again overlaid with 490 μL of solution 1 and centrifuged for 3 hours at 436,000 $\times$ *g*. The 490-μL top fraction was separated as the VLDL fraction (density $<$ 1.006 g/L). The bottom 980-μL fraction was transferred to a clean tube, and 490 μL of solution 2 was added. After ultracentrifugation at 436,000 $\times$ *g* for 3 hours, the 490-μL top fraction contained intermediate-density lipoprotein (IDL, 1.006 $<$ density $<$ 1.019 g/L). Using a similar procedure, LDL fractions (1.019 $<$ density $<$ 1.063 g/L) were separated by ultracentrifugation at 627,000 $\times$ g for 2 hours after the addition of solution 3. The high-density lipoprotein (HDL) fraction (1.063 $<$ density $<$ 1.210 g/L) was separated at 627,000 $\times$ *g* for 2 hours after the addition of solution 4 from the bottom fraction (density $>$1.210 g/L). The cholesterol content of each lipoprotein fraction was measured by the same method with the automatic analyzer (7170, Hitachi), as described above.

Statistical Analysis

Data were expressed as mean values and standard deviations (SD). Statistical analysis was performed by Dunnett's test or Student's paired *t* test (JMP; SAS Institute, Cary, NC,) against the control group or the pretreatment levels with a *P* value of less than .05 taken to indicate a statistically significant difference.

RESULTS

Hypolipidemic Action of S-2E and PRV in WHHL Rabbits

The homozygous WHHL rabbit is an animal model of familial hypercholesterolemia, having elevated levels of TC and TG. Indeed, homozygous WHHL rabbits used in the control group exhibited elevated levels of TC and TG (Fig 2). TC and TG levels in the WHHL rabbits (N = 11) were maintained from at least 2 months to 4 months after birth, as shown in Fig 2. From 3 months (0 week), the animals were given S-2E orally at doses of 30, 100, and 300 mg/kg for 4 weeks. We examined whether the S-2E treatment decreased the blood TC or TG levels, or both. As shown in Fig 3A,

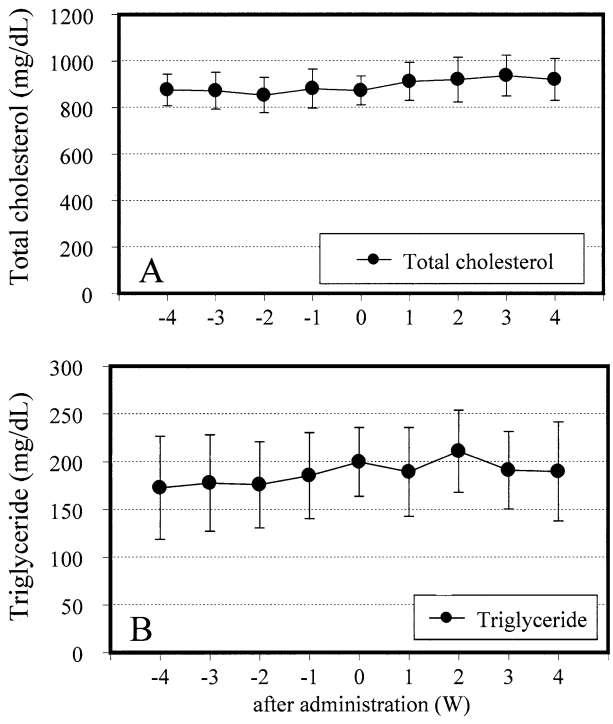

Fig 2. Changes in serum (A) TC and (B) TG in WHHL rabbits by S-2E administration. WHHL rabbits (N = 11) underwent a pretreatment period (4 weeks) and a treatment period (4 weeks). Blood was collected once per week and serum lipids were measured. Data are expressed as means and SD.

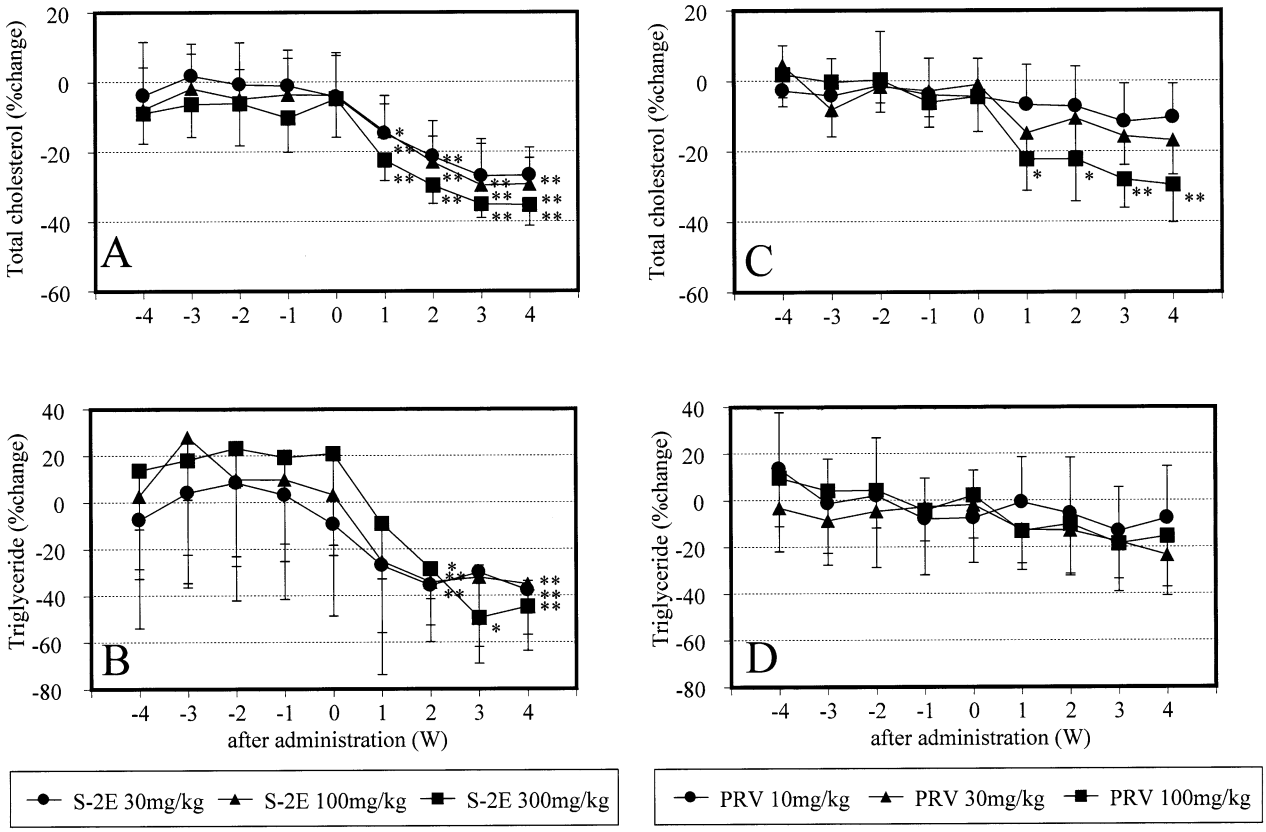

Fig 3. Changes in serum TC and TG in WHHL rabbits by S-2E and pravastatin administration. WHHL rabbits were divided into 4 groups (control group [n = 6], S-2E 30 mg/kg group [n = 7], S-2E 100 mg/kg group [n = 7], and S-2E 300 mg/kg group [n = 7]). HMPC or S-2E was given orally to WHHL rabbits. Blood was collected once per week and serum lipids were measured during the pretreatment period (4 weeks) and the S-2E-treatment period (4 weeks). Data are expressed as % change and SD against the control group. (A) Serum TC. (B) Serum TG. Using the same experimental protocol, PRV was given orally to WHHL rabbits (control group, PRV 10 mg/kg group, PRV 30 mg/kg group, and PRV 100 mg/kg group) (all groups: n = 5). Blood was collected once per week and measured for lipids in the obtained serum, during the pretreatment period (4 weeks) and the PRV treatment period (4 weeks). (C) Serum TC. (D) Serum TG. Significant difference in comparison for the control group were evaluated by Dunnett's test: *$P < .05$, **$P < .01$. For reasons of clarity, the results of Student's t test are not given.

S-2E significantly lowered the serum levels of TC at 1 week in a dose-dependent manner. Decreases in the TC levels induced by the S-2E treatment reached a maximum at 3 to 4 weeks, and the reduction rate of TC levels by S-2E at the doses of 30, 100, and 300 mg/kg after 4 weeks of treatment were 26.7% ± 8.0%, 29.3% ± 7.6%, and 35.3% ± 5.9%, respectively, compared with the control group. Furthermore, when compared with pretreatment levels of the same animals, S-2E significantly lowered serum TC levels, at least, after 2 weeks. Similar to the effect on TC levels, S-2E significantly lowered serum levels of TG at 2 weeks (Fig 3B), and this also reached maximum inhibition at 3 to 4 weeks. The reduction rates of TG levels by S-2E at the doses of 30, 100, and 300 mg/kg after 4 weeks of treatment were 37.6% ± 16.0%, 35.0% ± 12.0%, and 44.6% ± 19.0%, respectively. Moreover, when compared with the pretreatment levels of the same animals, S-2E significantly lowered serum TG levels from 1 week, except for 3 weeks at 30 mg/kg. The results clearly indicated that S-2E treatment lowered both serum TC and TG levels.

To compare the hypolipidemic effects of S-2E with those of PRV, the animals were given PRV orally at doses of 10, 30, and 100 mg/kg for 4 weeks. Figure 3C and D shows the changes in the serum levels of TC and TG in the vehicle-treated control group and those in the PRV-treated groups. It was found that the PRV treatment at 100 mg/kg significantly lowered TC levels to an extent similar to the S-2E treatment group (100 mg/kg) at 1 to 4 weeks (4 weeks: 29.5% ± 10.6%), compared with the control group (Fig 3C). Furthermore, when compared with the pretreatment levels of the same animals, PRV significantly lowered serum TC levels at 30 and 100 mg/kg from 1 week to 4 weeks. However, it showed no inhibitory effects on TG levels by 4 weeks in all experiments, compared with the control group, although PRV significantly changed the serum TG levels at some individual points within that of periods, when compared with the pretreatment levels in the same animals (Fig 3D).

Effect of S-2E on Lipoprotein Cholesterol Levels

To analyze in greater detail the changes in serum lipoprotein cholesterol levels following S-2E treatment, the serum of the

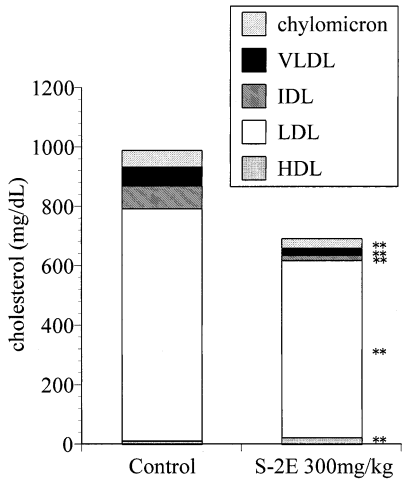

Fig 4. Changes in the serum lipoprotein cholesterol levels of WHHL rabbits with S-2E administration. After oral administration for 4 weeks, serum lipoproteins in the control group (n = 6) and S-2E 300 mg/kg group (n = 7) were fractionated by ultracentrifugation. Data are shown as the mean values of each lipoprotein. Significant differences in comparison for the control group were evaluated by Dunnett's test: *$P < .05$, **$P < .01$.

control group and the S-2E 300 mg/kg group, which lowered TC and TG levels most potently, sampled at 4 weeks after the start of administration, was subjected to lipoprotein fractionation by ultracentrifugation. In the S-2E–treated group, cholesterol levels in chylomicron, VLDL, IDL, and LDL fractions were significantly reduced (Fig 4). Of these fractions, the cholesterol content of the IDL and VLDL fractions was suppressed very potently by 77.1% ± 8.9% and 63.1% ± 10.0%, respectively, followed by the chylomicron fraction (42.4% ± 16.2%), and then the LDL fraction (23.7% ± 9.9%), while the HDL fraction approximately doubled. These results showed that the decrease in TC levels in the S-2E–treated groups was based on the significant decrease in the chylomicron, VLDL, IDL, and LDL fractions. Furthermore, these data showed that the decreased levels of VLDL particles led to decreases of IDL particles and LDL particles.

DISCUSSION

Inhibition of the synthesis of both sterols and fatty acids is an important target to improve mixed hyperlipidemia in the LDL receptor–deficient state. The present study was conducted to clarify whether such inhibitor(s) could lower the blood cholesterol and TG levels even in an LDL receptor–deficient state, for example, in homozygous WHHL rabbits, which are an LDL receptor–deficient animal model for human familial hypercholesterolemia.[6,7] We used a novel benzoic acid derivative, S-2E, which inhibits the synthesis of sterols and fatty acids in rat liver slices by noncompetitive inhibition of HMG-CoA reductase and acetyl-CoA carboxylase.[11] S-2E lowered blood TC levels and TG levels in homozygous WHHL rabbits at a dose of 30 mg/kg (Fig 3). Therefore, inhibitor(s) of synthesis of both sterols and fatty acids including S-2E may improve human familial hypercholesterolemia and mixed hyperlipidemia even in the LDL receptor–deficient state.

HMG-CoA reductase inhibitors cause the lipid-lowering effects by the induction of the hepatic LDL receptor[13,14] and by the decrease of VLDL secretion from the liver as secondary responses to the inhibition of cholesterol biosynthesis.[15-17] Furthermore, the TG-lowering effect of HMG-CoA reductase inhibitors may be dependent on the increase of LDL receptor–mediated clearance of VLDL particles, rather than the reduction of hepatic secretion of VLDL particles.[18] These data suggest that in homozygous WHHL rabbits, which exhibit impaired clearance of lipoproteins by mediating defective LDL receptor,[6,7] HMG-CoA reductase inhibitors have little or no effects, in particular on blood TG. Indeed, it has been reported that HMG-CoA reductase inhibitors including PRV did not alter the blood TG levels.[19-24] Similar results were observed in the present study (Fig 3). Therefore, the mechanism through which S-2E lowred the blood TC and TG levels seems to be independent of the LDL receptor–mediated pathway.

Blood lipid abnormalities in the LDL receptor–deficient state may be improved by suppressing MTP activity or through peroxisome proliferator–activated receptor (PPAR)−α. Indeed, MTP inhibitors reduced the blood cholesterol and TG levels by lowering VLDL particle secretion in homozygous WHHL rabbits, but caused hepatic TG accumulation.[8,9] In contrast, S-2E treatment did not change hepatic lipid levels, including TG, in Zucker fatty rats, although it lowered blood TC and TG levels.[11] Therefore, the lipid-lowering mechanism of S-2E seems to be different from that of MTP inhibitors. While it is known that fibrate derivatives such as bezafibrate express lipid-lowering effects by activating PPAR-α,[25-27] it has been reported that bezafibrate was not effective in homozygous WHHL rabbits.[18] Furthermore, similar results by bezafibrate treatment even at 300 mg/kg for 4 weeks were observed in our study (reduction rate after 4 weeks: TC by −5.2% ± 10.7%, TG by −13.8% ± 27.8%, unpublished data). Therefore, it is thought that the lipid-lowering effect of S-2E may not be mediated by PPAR-α activation, unlike that of fibrate derivatives.[25-27]

The anti-atherogenic lipoprotein, HDL particle, plays an essential role in the transport of excess cholesterol from extrahepatic tissues, for example, atherosclerotic lesions, to the liver,[28] resulting in reduction of the risk factor of coronary artery disease. In the present study, lipoprotein analysis showed that S-2E significantly increased HDL-C levels at 300 mg/kg. This finding suggests the possibility that S-2E may be able to increase HDL-C levels. Further analysis is necessary to clarify this possibility, using rats, which are another species, beside humans, in which the antihyperlipidemic agents predictably elevate HDL-C levels.

Hepatic lipid levels such as cholesterol, fatty acid, and TG in homozygous WHHL rabbits may be basically dependent on the biosynthetic potencies of their lipids in liver, because the rabbits exhibit impaired uptake of IDL, LDL, and VLDL particles into liver by the defective LDL receptor.[6,7] When the biosynthesis of hepatic lipids is limited, the formation of the VLDL particles is suppressed.[5] These facts suggest that S-2E may suppress the production of VLDL particles by limiting the synthesis of their lipids, because S-2E showed inhibitory activity on synthesis of sterols and fatty acids in rat liver

slices.[10,11] In fact, S-2E decreased the cholesterol contents of VLDL fraction and blood TG levels in homozygous WHHL rabbits (Fig 4). Furthermore, our previous study showed that S-2E lowered blood TC and TG levels by suppressing the new production of VLDL particles in Zucker fatty rats,[11] which exhibit hyperlipidemia by overproduction of VLDL particles.[29] Therefore, it is thought that S-2E may lower blood TC and TG levels even in an LDL receptor–deficient state by suppressing VLDL particle production.

Furthermore, S-2E reduced the cholesterol content of chylomicron fraction (Fig 4). This finding suggests the possibility that S-2E may influence the production of chylomicron particles in the intestine. However, the chylomicron lipid components are basically of dietary origin in the intestine, unlike VLDL particles in the liver.[30] It has been reported that human crypt intestinal epithelial cells and rat intestine are capable of cholesterol synthesis,[31,32] and the hamster intestine can synthesize cholesterol and fatty acids.[33] It is therefore reasonable to assume that S-2E may partly suppress chylomicron formation in the intestine by limiting the synthesis of necessary lipid components.

In conclusion, S-2E lowered TC and TG levels in the blood simultaneously, unlike PRV, in homozygous WHHL rabbits. Thus, S-2E may be useful to improve blood lipid abnormalities in the LDL receptor–deficient state.

ACKNOWLEDGMENT

We thank Dr Hidekazu Miyake for advice regarding this work and Dr Masashi Shiomi of Kobe University School of Medicine for technical advice. The authors are indebted to Professor J. Patrick Barron and Assistant Professor Raoul Breugelmans of the International Medical Communications Center of Tokyo Medical University for their review of the manuscript.

REFERENCES

1. McKenney JM: Pharmacotherapy of dyslipidemia. Cardiovasc Drugs Ther 15:413-422, 2001

2. Ballantyne CM: Low-density lipoproteins and risk for coronary artery disease. Am J Cardiol 82:3Q-12Q, 1998

3. Ucar M, Mjorndal T, Dahlqvist R: HMG-CoA reductase inhibitors and myotoxicity. Drug Safety 22:441-457, 2000

4. Wetterau JR, Aggerbeck LP, Bouma ME, et al: Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science 258:999-1001, 1992

5. Olofsson SO, Aap L, Boren J: The assembly and secretion of apolipoprotein B-containing lipoproteins. Curr Opin Lipidol 10:341-346, 1999

6. Wetterau JR, Gregg RE, Harrity TW, et al: An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. Science 282:751-754, 1998

7. Shiomi M, Ito T: MTP inhibitor decreases plasma cholesterol levels in LDL receptor-deficient WHHL rabbits by lowering the VLDL secretion. Eur J Pharmacol 431:127-131, 2001

8. Tanzawa K, Shimada Y, Kuroda M, et al: WHHL rabbit: a low density lipoprotein receptor-deficient animal model for familial hypercholesterolemia. FEBS Lett 118:81-84, 1980

9. Kita T, Brown MS, Watanabe Y, et al: Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbits, an animal model of familial hypercholesterolemia. Proc Natl Acad Sci USA 78:2268-2272, 1981

10. Ohno T, Yano S, Yamada H, et al: Synthesis of the optical isomers of 4-[1-(4-*tert*-butylphenyl)-2-oxo-4-pyrrolidine-4-yl]methyloxybenzoic acids (S-2) and their biological evaluation as antilipidemic agent. Chem Pharm Bull 47:1549-1554, 1999

11. Ohmori K, Yamada H, Yasuda A, et al: Anti-hyperlipidemic action of a newly synthesized benzoic acid derivative, S-2E. Eur J Phamacol 471:69-76, 2003

12. Hatch FT, Lees RS: Practical method for plasma lipoprotein analysis. Adv Lipid Res 6:1-68, 1968

13. Bilheimer DW, Grundy SM, Brown MS, et al: Mevilonin and colestipol stimulate receptor-mediated clearance of low density lipoprotein from plasma in familial hypercholesterolemia. Proc Natl Acad Sci USA 90:80-84, 1983

14. Reiher E, Rudling M, Stahhlberg D, et al: Influence of pravastatin, a specific inhibitor of HMG-CoA reductase, on hepatic metabolism of cholesterol. N Engl J Med 332:224-228, 1990

15. Ginsberg HN, Le NA, Short MP: Suppression of apolipoprotein B production during treatment of cholesteryl ester storage disease with lovastatin. Implications for regulation of apolipoprotein B synthesis. J Clin Invest 80:1692-1697, 1987

16. Arad Y, Ramakrishnan R, Ginsberg HN: Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: Implications of the pathophysiology of apoB production. J Lipid Res 31:567-582, 1990

17. Bakker-Arkema RD-G, Davidson MH, Goldstein RJ, et al: Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. JAMA 275:128-133, 1996

18. Hiyoshi H, Yanagimachi M, Ito M, et al: Squalene synthase inhibitors reduce plasma triglyceride through a low-density lipoprotein receptor-independent mechanism. Eur J Pharmacol 23:345-352, 2001

19. Tsujita Y, Kuroda M, Shimada Y, et al: CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: Tissue-selective inhibitor of sterol synthesis and hypolipidemic effect on various animal species. Biochim Biophys Acta 877:50-60, 1986

20. Watanabe Y, Ito T, Saeki M, et al: Hypolipidemic effects of CS-500 (ML-236B) in WHHL rabbits, a heritable animal model of hyperlipidemia. Atherosclerosis 38:27-81, 1981

21. Khachadurian AK, Shimamura T, Rozovski SJ, et al: Pravastatin decreases serum lipids and vascular cholesterol deposition in Watanabe heritable hyperlipidemic (WHHL) rabbits. Jpn Heart J 32:675-685, 1991

22. Shiomi M, Ito T: Pravastatin sodium, a competitive inhibitor of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase, decreases the cholesterol content of newly secreted very-low-density lipoprotein in Watanabe heritable hyperlipidemic rabbits. Metabolism 43:559-564, 1994

23. Shiomi M, Ito T: Effect of cerivastatin sodium, a new inhibitor of HMG-CoA reductase, on plasma lipid levels, progression of atherosclerosis, and the lesional composition in the plaques of WHHL rabbits. Br J Pharmacol 126:961-968, 1999

24. Shiomi M, Ito T, Tsukada T, et al: Combination treatment with troglitazone, an insulin action enhancer, and pravastatin, an inhibitor of HMG-CoA reductase, shows a synergistic effect on atherosclerosis of WHHL rabbits. Atherosclerosis 142:345-353, 1999

25. Staels B, Dallongeville J, Aurwerx J, et al: Mechanism of action of fibrates on lipid and lipoprotein metabolism. Circulation 98:2088-2093, 1998

26. Linton MF, Fazio S: Re-emergence of fibrates in the management of dyslipidemia and cardiovascular risk. Curr Atheroscler Rep 2:29-35, 2000

27. Dana SL, Hoener PA, Bilakovs JM, et al: Peroxisome proliferator-activated receptor subtype-specific regulation of hepatic and peripheral gene expression in Zucker diabetic fatty rats. Metabolism 50:963-971, 2001

28. Kwiterovich PO Jr: The antiatherogenic role of high-density lipoprotein cholesterol. Am J Cardiol 82:13Q-21Q, 1998

29. Bray GA: The Zucker fatty rat: A review. Fed Proc 36:148-153, 1977

30. Cartwright IJ, Plonnè D, Higgins JA: Intracellular events in the assembly of chylomicrons in rabbit enterocytes. J Lipid Res 41:1728-1739, 2000

31. Levy E, Beaulieu J-F, Delvin E, et al: Human crypt intestinal epithelial cells are capable of lipid production, apolipoprotein synthesis, and lipoprotein assembly. J Lipid Res 41:12-22, 2000

32. Hara H, Haga S, Aoyama Y, et al: Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. J Nutr 129:942-948, 1999

33. Field FJ, Born E, Mathur SN: Fatty acid flux suppresses fatty acid synthesis in hamster intestine independently of sterol regulatory element-binding protein-1 expression. J Lipid Res 44:1199-1208, 2003