Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 466 (2003) 155–161
www.elsevier.com/locate/ejphar

Lipid-lowering effects of TAK-475, a squalene synthase inhibitor, in animal models of familial hypercholesterolemia

Yuichiro Amano, Tomoyuki Nishimoto, Ryu-ichi Tozawa, Eiichiro Ishikawa, Yoshimi Imura*, Yasuo Sugiyama

Pharmacology Research Laboratories II, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 2-17-85, Juso-Honmachi, Yodogawa, Osaka 532-8686, Japan

Received 12 September 2002; received in revised form 21 February 2003; accepted 25 February 2003

Abstract

The lipid-lowering effects of 1-{2-[(3*R*,5*S*)-1-(3-acetoxy-2,2-dimethylpropyl)-7-chloro-1,2,3,5-tetrahydro-2-oxo-5-(2,3-dimethoxyphenyl)-4,1-benzoxazepine-3-yl] acetyl} piperidin-4-acetic acid (TAK-475), a novel squalene synthase inhibitor, were examined in two models of familial hypercholesterolemia, low-density lipoprotein (LDL) receptor knockout mice and Watanabe heritable hyperlipidemic (WHHL) rabbits. Two weeks of treatment with TAK-475 in a diet admixture (0.02% and 0.07%; approximately 30 and 110 mg/kg/day, respectively) significantly lowered plasma non-high-density lipoprotein (HDL) cholesterol levels by 19% and 41%, respectively, in homozygous LDL receptor knockout mice. The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, simvastatin and atorvastatin (in 0.02% and 0.07% admixtures), also reduced plasma levels of non-HDL cholesterol. In homozygous WHHL rabbits, 4 weeks of treatment with TAK-475 (0.27%; approximately 100 mg/kg/day) lowered plasma total cholesterol, triglyceride and phospholipid levels by 17%, 52% and 26%, respectively. In Triton WR-1339-treated rabbits, TAK-475 inhibited to the same extent the rate of secretion from the liver of the cholesterol, triglyceride and phospholipid components of very-low-density lipoprotein (VLDL). These results suggest that the lipid-lowering effects of TAK-475 in WHHL rabbits are based partially on the inhibition of secretion of VLDL from the liver. TAK-475 had no effect on plasma aspartate aminotransferase and alanine aminotransferase activities. Thus, the squalene synthase inhibitor TAK-475 revealed lipid-lowering effects in both LDL receptor knockout mice and WHHL rabbits.

Keywords: TAK-475; Squalene synthase inhibitor; Hypercholesterolemia, familial; LDL receptor knockout, mouse; WHHL, rabbit

1. Introduction

It is well known that elevated plasma cholesterol is a major risk factor for atherosclerosis and coronary heart disease (Slack, 1969). Familial hypercholesterolemia is a genetic disease characterized by low-density lipoprotein (LDL) receptor deficiency (Hobbs et al., 1992). The most common form of familial hypercholesterolemia is heterozygous familial hypercholesterolemia, which usually leads to premature coronary heart diseases before the age of 60 years. Homozygous familial hypercholesterolemia is a much rarer condition, but the effects are more sever than those of the heterozygous form (Allen et al., 1980). Patients with homozygous familial hypercholesterolemia present with marked hypercholesterolemia from birth, experience coronary heart diseases in childhood and rarely survive beyond the age of 30 years (Dammerman and Breslow, 1995). The therapy for familial hypercholesterolemia patients with severe hypercholesterolemia is LDL apheresis. Combined treatment with LDL apheresis and lipid-lowering agents such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) may be used if the intervention with LDL apheresis alone does not achieve target LDL cholesterol levels (Jacob et al., 1993; Geiss et al., 1999). Although, at high doses (40–80 mg/man), the HMG-CoA reductase inhibitor atorvastatin decreases plasma cholesterol levels in patients with homozygous familial hypercholesterolemia, the lipid-lowering agents (including the statins) that are currently available are not effective enough to achieve control of plasma LDL-cholesterol levels (Geiss et al., 1999).

* Corresponding author. Tel.: +81-6-6300-6876; fax: +81-6-6300-6306.
E-mail address: Imura_Yoshimi@takeda.co.jp (Y. Imura).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01549-8

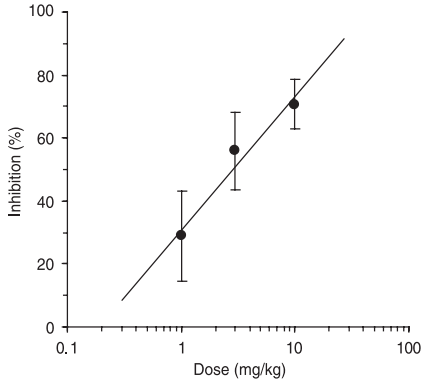

Fig. 1. Effects of TAK-475 on de novo hepatic cholesterol synthesis in LDL receptor knockout mice. TAK-475 (1, 3 and 10 mg/kg) suspended in a 0.5% methylcellulose solution was administered orally to 10-week-old female LDL receptor konockout mice while they were in a nonfasted state ($n = 5–6$). One hour after the drug administration, sodium [^{14}C]acetate (50 μCi/kg) was injected intravenously into the mice, which were then sacrificed a further 1 h later. Their livers were quickly isolated and saponified. The sterol fractions were extracted with petroleum ether under alkaline conditions and the digitonin-precipitable sterols were measured by a liquid scintillation counter. Values are means ± S.E.M. The half-maximum effective concentration (ED_{50}) value was 2.8 mg/kg, administered orally (0.65–9.0 mg/kg, 95% confidence interval).

Statins, the most common lipid-lowering agents, reduce cholesterol biosynthesis through inhibition of the conversion of HMG-CoA to mevalonic acid, the early and rate-limiting stage in cholesterol biosynthesis, resulting in an increase in the expression of LDL receptors. By inhibiting HMG-CoA reductase, statins also decrease the levels of intermediate metabolites of cholesterol biosynthesis such as isoprenylates, ubiquinones and dolichol, and this may be the cause of myopathy, a common adverse effect of the statins (Ghirlanda et al., 1993; Hsu et al., 1995). Squalene synthase inhibitors, on the other hand, reduce cholesterol biosynthesis through inhibition of the conversion of farnesyl pyrophosphate to squalene without decreasing the levels of isoprenylates, ubiquinones and dolichol, so that squalene synthase inhibitors are less likely to cause adverse effects resulting from lack of the intermediate metabolites of cholesterol biosynthesis (Rosenberg, 1998). It has been reported that squalene synthase inhibitors exert plasma cholesterol-lowering effects in rats, guinea pigs, hamsters, common marmosets, and rhesus monkeys, and both plasma cholesterol- and triglyceride-lowering effects in high-fat fed hamsters and common marmosets (Baxter et al., 1992; Amin et al., 1997; Hiyoshi et al., 2000; Ugawa et al., 2000). Thus, the inhibition of squalene synthase might represent an attractive new approach for the treatment of patients with familial hypercholesterolemia.

1-{2-[(3*R*,5*S*)-1-(3-Acetoxy-2,2-dimethylpropyl)-7-chloro-1,2,3,5-tetrahydro-2-oxo-5-(2,3-dimethoxyphenyl)-4,1-benzoxazepine-3-yl] acetyl} piperidin-4-acetic acid (TAK-475) is a novel and potent squalene synthase inhibitor (Miki et al., 2002). It has been reported that TAK-475 lowers plasma cholesterol levels in hypertriglycemic Wistar fatty rats, dogs, cynomolgus monkeys and common marmosets (Nishimoto et al., unpublished observations). In the study presented here, we examined the lipid-lowering effects of TAK-475 in two models of familial hypercholesterolemia, homozygous LDL receptor knockout mice and homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits. Homozygous LDL receptor knockout mice, which completely lack LDL receptors, were first developed by Ishibashi et al. (1993). These mice gradually develop hyperlipidemia when fed with an atherogenic diet and finally develop aortic atherogenic lesions (Ishibashi et al., 1994; Magoulas et al., 1997). WHHL rabbits have partially effective LDL receptors

Table 1
Lipid-lowering effects of TAK-475, simvastatin and atorvastatin in LDL receptor knockout mice

Drugs	Dose	Total cholesterol		Non-HDL cholesterol		Triglyceride	
		mg/dl	% of initial	mg/dl	% of initial	mg/dl	% of initial
Experiment 1							
Control	–	223 ± 9	102 ± 6	158 ± 8	120 ± 10	94 ± 14	87 ± 14
TAK-475	0.02%	199 ± 12	92 ± 8	138 ± 10	107 ± 13	93 ± 8	87 ± 12
TAK-475	0.07%	166 ± 9	75 ± 3[a]	103 ± 8	77 ± 7[a]	64 ± 6	60 ± 8
Simvastatin	0.02%	206 ± 11	94 ± 7	135 ± 10	102 ± 9	95 ± 8	88 ± 11
Simvastatin	0.07%	190 ± 14	84 ± 1[b]	113 ± 13	79 ± 3[a]	87 ± 8	76 ± 5
Experiment 2							
Control	–	236 ± 13	103 ± 5	173 ± 12	102 ± 6	86 ± 12	88 ± 12
TAK-475	0.02%	197 ± 5	89 ± 2[a]	139 ± 5	83 ± 4[a]	87 ± 10	93 ± 19
TAK-475	0.07%	156 ± 2	68 ± 3[a]	98 ± 2	60 ± 3[a]	63 ± 6	57 ± 8
Atorvastatin	0.02%	186 ± 9	81 ± 4[a]	128 ± 5	78 ± 4[a]	75 ± 8	79 ± 14
Atorvastatin	0.07%	135 ± 7	59 ± 3[a]	75 ± 8	45 ± 4[a]	80 ± 4	78 ± 10

In both Experiments 1 and 2, each drug was administered as a diet admixture (0.02% and 0.07%; approximately 30 and 110 mg/kg/day, respectively) to 13-week-old female LDL receptor knockout mice for 2 weeks. Plasma lipid levels were measured by using a biochemical autoanalyzer. Values are means ± S.E.M. ($n = 5–6$). Statistical comparisons were made using the percentage change from the initial values (% of initial).

[a] $P \leq 0.025$ vs. the control values (one-tailed Williams' test for each compound separately).

[b] $P \leq 0.025$ vs. the control values (one-tailed Shirley–Williams test).

and develop atherosclerotic lesions when fed chow diets (Watanabe, 1980; Kita et al., 1981; Watanabe et al., 1988; Shiomi et al., 1994).

2. Materials and methods

2.1. Materials

TAK-475 was synthesized in our laboratories. Atorvastatin (Lipitor) was purchased from Warner-Lambert. Simvastatin, all reagents for biochemical autoanalysis and high-density lipoprotein (HDL) cholesterol precipitation reagents were purchased from Wako Pure Chemical (Osaka, Japan). Triton WR-1339 was purchased from Ruqer Chemical (USA).

2.2. LDL receptor knockout mice

Breeding pairs of homozygous LDL receptor knockout mice were obtained from Jackson Laboratories and a colony was maintained at Takeda Chemical Industries. Mice were given rodent chow (CE-2; Crea, Japan) and water ad libitum, and were housed in a temperature- and humidity-controlled room.

The inhibition of de novo hepatic cholesterol biosynthesis in these mice was determined by measuring the conversion of intravenously injected sodium [^{14}C]acetate to cholesterol (Tsujita et al., 1986). TAK-475 (1, 3 and 10 mg/kg) suspended in a 0.5% methylcellulose vehicle solution was administered orally to 10-week-old female LDL receptor knockout mice in a non-fasted state (10 ml/kg). The control group was given an equal volume of the vehicle. One hour after the administration, sodium [^{14}C]acetate (50 μCi/kg) was injected intravenously into the mice. Mice were sacrificed a further 1 h after the injection of sodium [^{14}C]acetate. Their livers were quickly isolated, saponified in the presence of 90% KOH and 99% ethanol at 100 °C for 4 h, and sterol was extracted with petroleum ether under alkaline conditions. The petroleum ether extracts were dried under N_2 gas and the precipitates were dissolved in a 1:1 ethanol/acetone solution. The sterol fraction was precipitated with 0.5% digitonin solution in 50% ethanol. The radioactivities of the precipitated fractions were measured by a liquid scintillation counter (WALLAC LSC-1414) with Triton X-100/toluene-based scintillation cocktail. Half-maximal effective concentration (ED_{50}) values were determined by linear regression of the inhibition of cholesterol synthesis versus the dose of drug.

To examine the lipid-lowering effects of TAK-475, 13-week-old female homozygous LDL receptor knockout mice were used. Blood samples were collected using heparin as an anticoagulant, while animals were in a non-fasted state, every week for 2 weeks. These samples were immediately placed on ice and separated by centrifugation to obtain plasma samples. Plasma total cholesterol and triglyceride levels were measured enzymatically by using a biochemical autoanalyzer (Hitachi Autoanalyzer 7070, Japan). HDL cholesterol was separated out by chemical precipitations of apolipoprotein-B-containing lipoproteins with heparin and Mn^{2+} and then determined enzymatically. Non-HDL cholesterol was estimated as the difference between total cholesterol and HDL cholesterol. Mice were divided into five groups for each experiment. TAK-475, simvastatin and atorvastatin were administered orally to LDL receptor knockout mice ($n = 5–6$) as diet admixtures (0.02% and 0.07% for each drug; approximately 30 and 110 mg/kg/day, respectively) for 2 weeks. All animal experiments were performed according to the guidelines of the Takeda Experimental Animal Care and Use Committee.

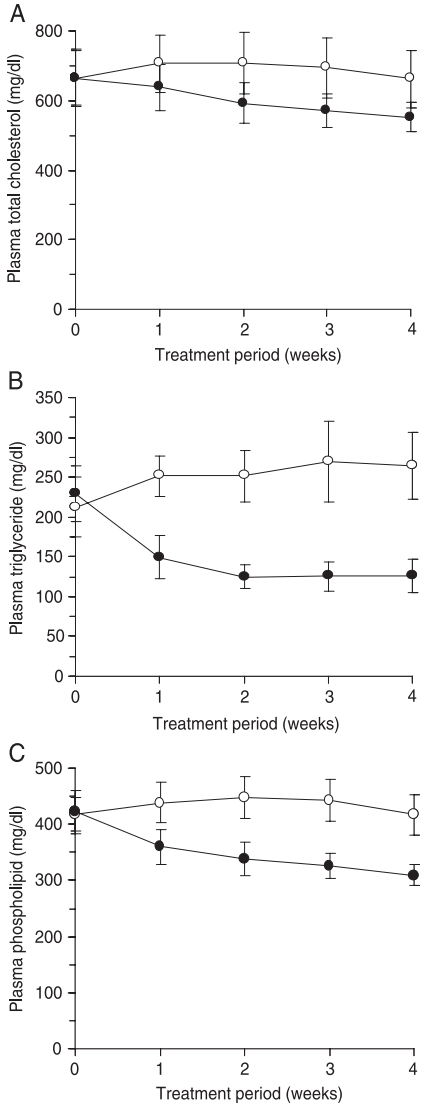

Fig. 2. Effects of TAK-475 on plasma lipid levels in WHHL rabbits. TAK-475 was administered as a 0.27% diet admixture (approximately 100 mg/kg/day) to 6-month-old WHHL rabbits ($n = 6$). (A) Total cholesterol, (B) triglyceride and (C) phospholipid. Plasma lipid levels were measured once each week by using a biochemical autoanalyzer. Values are means ± S.E.M. Open circles, control; closed circles, TAK-475.

2.3. WHHL rabbits

Twelve homozygous WHHL rabbits (four males and eight females) aged 6 months were purchased from Kitayama Labes (Nagano, Japan). The rabbits were given a standard rabbit chow (RC-4; Oriental Yeast, Tokyo, Japan) and water ad libitum, and were housed in a temperature- and humidity-controlled room. Blood samples were collected from a central ear artery with heparin as an anticoagulant, while the animals were in a non-fasted state, every week. These samples were immediately placed on ice and separated by centrifugation to obtain plasma samples. Plasma parameters (total cholesterol, triglyceride, phospholipid, aspartate aminotransferase and alanine aminotransferase) were measured enzymatically with a biochemical autoanalyzer (Hitachi Autoanalyzer 7070). Rabbits were divided into two groups: a control group ($n=6$, two males and four females) and a treated group ($n=6$, two males and four females). During the treatment periods, rabbits were fed with 120 g of diet/day every evening. TAK-475 mixed with the pellet diet (0.27%; corresponding to 100 mg/kg/day) was given for 4 weeks.

To investigate the effects of TAK-475 on hepatic lipid secretion, Triton WR-1339 was used to block the clearance of plasma triglyceride by interfering with the action of lipoprotein lipase (Hornick et al., 1983; Nagata and Zilverssmit, 1987). On the day following completion of the 4-week treatment with TAK-475, Triton WR-1339 (400 mg/2 ml in saline) was injected into WHHL rabbits through an ear vein while they were in a non-fasted state (2 ml/kg). Blood was collected from the ear artery before and 6 h after the Triton WR-1339 injection. Plasma samples were separated into lipoprotein fractions using a high-performance liquid chromatography (HPLC) system (Brousseau et al., 1997). A 100-μl sample of plasma from each individual rabbit was applied to the HPLC system (LC-10 system; Shimadzu, Tokyo, Japan), which utilizes a Superose 6 column (Pharmacia Biotech, USA). Lipoproteins were eluted at 0.5 ml/min with elution buffer (150 mM NaCl, 10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid, 0.2 g/l NaN_3). After the initial 5.5 ml had been eluted, the next 15 ml was collected in 0.3-ml aliquots with a fraction collector (Model 2110, Bio Rad, USA). The lipid concentrations in each aliquot were measured using the biochemical autoanalyzer. Very-low-density lipoprotein (VLDL) and LDL lipids were estimated by calculating the area under the peak corresponding to VLDL and LDL, respectively. Secretion rates of hepatic cholesterol, triglyceride and phospholipid are represented as increments in plasma lipid levels per hour.

2.4. Statistical analysis

In LDL receptor knockout mice, comparison between the control group and the drug-treated groups was performed with one-tailed Williams' test for each compound separately. In WHHL rabbits, data for plasma parameters during the treatment period were analyzed by repeated-measures analysis of variance (ANOVA). The change from the baseline for all continuous variables was analyzed by ANOVA. Comparisons of lipoprotein lipid compositions and hepatic lipid secretion rates between the control group and the drug-treated groups were performed with Student's *t*-test. *P*-values less than 0.025, 0.05 and 0.05 were considered statistically significant by one-tailed Williams' test, ANOVA and Student's *t*-test, respectively. Data are presented as means ± S.E.M.

3. Results

3.1. Effect of TAK-475 on hepatic cholesterogenesis in LDL receptor knockout mice

TAK-475 (1, 3 and 10 mg/kg, administered orally) inhibited de novo hepatic cholesterol synthesis in a dose-dependent manner in female LDL receptor knockout mice. The ED_{50} value was estimated to be 2.8 mg/kg (Fig. 1).

Table 2
Effects of TAK-475 on lipid components of lipoproteins in WHHL rabbits

Drugs	Dose	VLDL (mg/dl)			LDL (mg/dl)		
		Cholesterol	Triglyceride	Phospholipid	Cholesterol	Triglyceride	Phospholipid
Control	–	124 ± 20	81 ± 26	73 ± 10	516 ± 70	133 ± 16	327 ± 35
TAK-475	0.27%	77 ± 11 (−38%)	28 ± 9 (−65%)	39 ± 5[a] (−46%)	457 ± 32 (−12%)	68 ± 6[b] (−49%)	260 ± 17 (−21%)

TAK-475 was administered as a 0.27% diet admixture (approximately 100 mg/kg/day) to 6-month-old homozygous WHHL rabbits ($n=6$) for 4 weeks. The plasma sample was separated into each lipoprotein fraction by using a HPLC system (Brousseau et al., 1997). A 100-μl sample of plasma from each individual rabbit was applied to the HPLC system (LC-10 system; Shimadzu), which utilizes a Superose 6 column (Pharmacia Biotech). Lipoproteins were eluted at 0.5 ml/min with elution buffer (150 mM NaCl, 10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid, 0.2 g/l NaN_3). After the initial 5.5 ml had been eluted, the next 15 ml was collected in 0.3-ml aliquots with a fraction collector (Model 2110, Bio Rad). The lipid concentrations in each aliquot were measured using a biochemical autoanalyzer. VLDL and LDL lipids were estimated by calculating the area under the peak corresponding to VLDL and LDL, respectively. Values are means ± S.E.M. Values in parentheses are percentage changes from the control values.

[a] $P \leq 0.05$ vs. the control values (Student's *t*-test).

[b] $P \leq 0.01$ vs. the control values (Student's *t*-test).

3.2. Effects of TAK-475 in LDL receptor knockout mice

In female LDL receptor knockout mice, TAK-475 at doses of 0.02% and 0.07% in the diet (approximately 30 and 110 mg/kg/day, respectively) lowered plasma non-HDL cholesterol significantly and dose-dependently by 19% and 41%, respectively (Table 1, Experiment 2). Simvastatin and atorvastatin also lowered these plasma lipids. TAK-475 tended to decrease plasma triglyceride in a dose-dependent manner, although significant effects were not observed. TAK-475 had no effects on plasma aspartate aminotransferase activity, even when administered at a higher dose (data not shown).

3.3. Effects of TAK-475 in WHHL rabbits

Homozygous WHHL rabbits exhibited hyperlipidemia at the age of 6 months and this hyperlipidemia was maintained during the experimental period (Fig. 2). Four weeks of treatment with TAK-475 at a dose of 0.27% in the diet (approximately 100 mg/kg/day) significantly lowered plasma total cholesterol levels by 17% (main effects of group: $F(1,10)=7.602$, $P \leq 0.05$ and time: $F(3,30)=13.241$, $P \leq 0.01$), triglyceride by 52% (main effect of group: $F(1,10)=39.106$, $P \leq 0.01$) and phospholipids by 26% (main effects of group: $F(1,10)=27.667$, $P \leq 0.01$, time: $F(3,30)=12.110$, $P \leq 0.01$ and group × time interaction: $F(3,30)=3.389$, $P \leq 0.05$) (Fig. 2). Submaximal reductions in plasma lipids were obtained at 2 weeks and sustained reductions continued throughout the treatment period. Furthermore, TAK-475 was more potent in reducing triglyceride than in reducing total cholesterol and phospholipid. At the end of the experiment, lipid components of the VLDL and LDL fractions were measured. TAK-475 significantly reduced cholesterol, triglyceride and phospholipid in the VLDL fraction by 38%, 65% and 46%, respectively, and triglyceride in the LDL fraction by 49% (Table 2). We then used Triton WR-1339 to assess the effects of TAK-475 on hepatic VLDL-triglyceride secretion. TAK-475 tended to inhibit the rate of secretion of triglyceride from the liver (Table 3). Hepatic VLDL-cholesterol and-phospholipid secretion rates were also inhibited by 16% and 20%, respectively (Table 3). TAK-475 had no effects on plasma aspartate aminotransferase and alanine aminotransferase activities or on body weight gain throughout the experimental period (Fig. 3).

Table 3
Effects of TAK-475 on the rate of secretion of VLDL components in WHHL rabbits

Drugs	Dose	Secretion rate (mg/dl/h)		
		Cholesterol	Triglyceride	Phospholipid
Control	–	27 ± 2	120 ± 14	31 ± 1
TAK-475	0.27%	23 ± 2	91 ± 13	25 ± 2[a]
		(−16%)	(−24%)	(−20%)

TAK-475 was administered as a 0.27% diet admixture (approximately 100 mg/kg/day) to 6-month-old homozygous WHHL rabbits ($n=6$) for 4 weeks. After the treatment period, Triton WR-1339 (400 mg/2 ml/kg) was injected into the rabbits and plasma lipid levels were measured before and 6 h after the injection. The plasma sample was separated into each lipoprotein fraction using a HPLC system (Brousseau et al., 1997). A 100-μl sample of plasma from each individual rabbit was applied to the HPLC system (LC-10 system; Shimadzu), which utilizes a Superose 6 column (Pharmacia Biotech). Lipoproteins were eluted at 0.5 ml/min with elution buffer (150 mM NaCl, 10 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid, 0.2 g/l NaN_3). After the initial 5.5 ml was eluted, the next 15 ml was collected in 0.3-ml aliquots with a fraction collector (Model 2110, Bio Rad). The lipid concentrations in each aliquot were measured using a biochemical autoanalyzer. VLDL lipids were estimated by calculating the area under the peak corresponding to VLDL. Values are means ± S.E.M. Values in parentheses are percentage changes from the control values.

[a] $P \leq 0.05$ vs. the control values (Student's t-test).

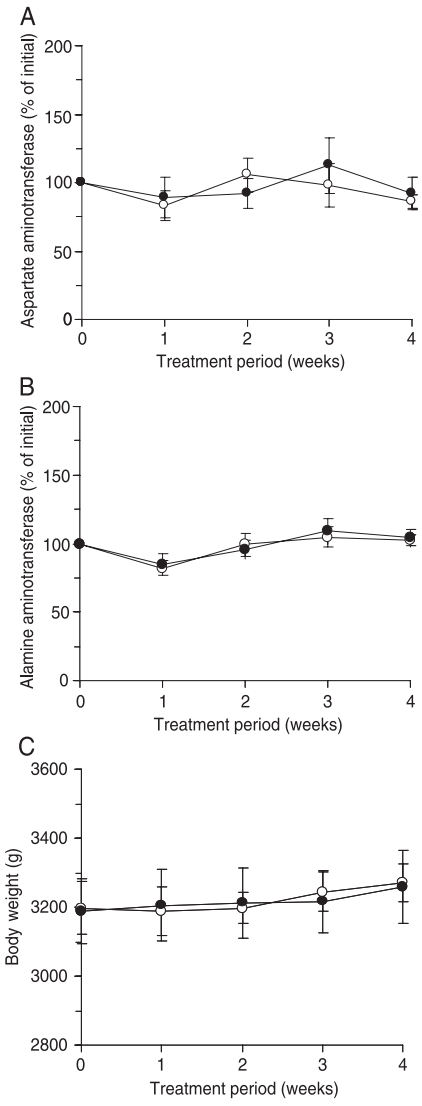

Fig. 3. Effects of TAK-475 on plasma aspartate aminotransferase and alanine aminotransferase activities and body weight in WHHL rabbits. TAK-475 was administered as a 0.27% diet admixture (approximately 100 mg/kg/day) to 6-month-old WHHL rabbits ($n=6$). (A) Aspartate aminotransferase, (B) alanine aminotransferase and (C) body weight. Plasma aspartate aminotransferase and alanine aminotransferase activities were measured once each week by using a biochemical autoanalyzer. Values are means ± S.E.M. Open circles, control; closed circles, TAK-475.

4. Discussion

The purpose of this study was to examine the lipid-lowering effects of TAK-475, a novel squalene synthase inhibitor, in two different models of familial hypercholesterolemia, LDL receptor knockout mice and WHHL rabbits.

TAK-475 significantly lowered plasma total and non-HDL cholesterol levels and tended to lower plasma triglyceride levels in homozygous LDL receptor knockout mice (Table 1). This is the first report of a squalene synthase inhibitor lowering plasma cholesterol levels in homozygous LDL receptor knockout mice. Simvastatin and atorvastatin also lowered plasma total and non-HDL cholesterol levels. It has been reported that atorvastatin significantly lowers plasma non-HDL cholesterol levels without decreasing apolipoprotein B and triglyceride secretion rates from the liver in LDL receptor knockout mice (Bisgaier et al., 1997). Our finding that atorvastatin lowered plasma non-HDL cholesterol levels confirms the results of Bisgaier et al. (1997). At higher clinical doses, atorvastatin reduces plasma cholesterol levels in patients with either heterozygous or homozygous familial hypercholesterolemia (Marais et al., 1997; Raal et al., 2000). Taken together, the present data suggest that treatment with TAK-475 may be effective for patients with either heterozygous or homozygous familial hypercholesterolemia. In LDL receptor knockout mice, oral TAK-475 inhibited de novo hepatic cholesterogenesis with an ED50 value of 2.8 mg/kg (Fig. 1). Inhibition of hepatic cholesterogenesis may contribute to the lipid-lowering effects of TAK-475 in LDL receptor knockout mice, although pharmacokinetic parameters were not determined in the study reported here.

TAK-475 significantly lowered plasma total cholesterol, triglyceride and phospholipid levels by 17%, 52% and 26%, respectively, in homozygous WHHL rabbits (Fig. 2). In particular, a marked reduction of plasma triglyceride level was observed after treatment with TAK-475; furthermore, the cholesterol, triglyceride and phospholipid contents of VLDL and LDL were also reduced by TAK-475 (Table 2). Hiyoshi et al. (2001) have reported that the squalene synthase inhibitor ER-28448 reduced only plasma triglyceride levels when administered intravenously to homozygous WHHL rabbits. Therefore, our studies provide the first report of a squalene synthase inhibitor reducing the levels of both plasma cholesterol and triglyceride in these LDL receptor-negative or -defective conditions. After treatment of WHHL rabbits with Triton WR-1339, TAK-475 reduced the secretion rate of VLDL-cholesterol, -triglyceride and -phospholipid by 16%, 24% and 20%, respectively (Table 3). Thus, TAK-475 tended to reduce the secretion rate of VLDL particles from the liver. It has been reported that statins (pravastatin, fluvastatin, cerivastatin and atorvastatin) have shown moderate reductions of plasma triglyceride levels in WHHL rabbits (Shiomi and Ito, 1994, 1999; Shiomi et al., 1994; Arai et al., 1998). Pravastatin and fluvastatin reduce plasma cholesterol levels by reducing the cholesterol content of VLDL particles, not by reducing the rate of secretion of VLDL from the liver (Shiomi and Ito, 1994; Shiomi et al., 1994). Therefore, the inhibition of the secretion of VLDL particles might be the reason why TAK-475 dramatically reduced plasma triglyceride levels in WHHL rabbits. The mechanism underlying this difference between the effects of TAK-475 and of the statins is not clear. In in vitro experiments in HepG2 cells, atorvastatin enhanced the synthesis of both triglyceride and fatty acids, but TAK-475 had no, or only slight, effects on the synthesis of these lipids (Tozawa et al., unpublished observations). This difference might contribute to the divergent effects of TAK-475 and the statins on hepatic triglyceride secretion.

In conclusion, the effects of TAK-475, a novel squalene synthase inhibitor, were examined in two different animal models of familial hypercholesterolemia. TAK-475 significantly lowered plasma lipid levels in homozygous LDL receptor knockout mice and homozygous WHHL rabbits without affecting the activities of the hepatic enzymes.

Acknowledgements

We thank Drs. Z. Terashita and T. Wada for continuous advice and Ms. Y. Yamada for technical support. We also thank Drs. T. Miki, M. Kori, and H. Mabuchi for preparing the TAK-475.

References

Allen, J.M., Thompson, G.R., Myant, N.B., Steiner, R., Oakley, C.M., 1980. Cardiovascular complications of homozygous familial hypercholesterolemia. Br. Heart J. 44, 361–368.

Amin, D., Rutledge, R.Z., Needle, S.N., Galczenski, H.F., Neuenschwander, K., Scotese, A.C., Maguire, M.P., Bush, R.C., Hele, D.J., Bilder, G.E., Perrone, M.H., 1997. RPR 107393, a potent squalene synthase inhibitor and orally effective cholesterol-lowering agent: comparison with inhibitors of HMG-CoA reductase. J. Pharmacol. Exp. Ther. 281, 746–752.

Arai, Y., Funatsu, T., Kakuta, H., Tanaka, H., Yoshida, M., Hisaki, K., Usuta, S., 1998. Antiatherosclerotic effects of YM548 in Watanabe heritable hyperlipidemic rabbits. Jpn. Pharmacol. Ther. 26, 1475–1485.

Baxter, A., Fitzgerald, B.J., Hutson, J.L., McCarthy, A.D., Motteram, J.M., Ross, B.C., Sapra, M., Snowden, M.A., Watson, N.S., Williams, R.J., Wright, C., 1992. Squalestatin 1, a potent inhibitor of squalene synthase, which lowers serum cholesterol in vivo. J. Biol. Chem. 267, 11705–11708.

Bisgaier, C.L., Essenburg, A.D., Auerbach, B.J., Pape, M.E., Sekerke, C.S., Gee, A., Wolle, S., Newton, R.S., 1997. Attenuation of plasma low density lipoprotein cholesterol by select 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in mice devoid of low density lipoprotein receptors. J. Lipid Res. 38, 2502–2515.

Brousseau, M.E., Santamaaria-Fojo, S., Vaisman, B.L., Applebaum-Bowden, D., Berrard, A.M., Talley, G.D., Brewer Jr., B., Hoeg, J.M., 1997. Overexpression of human lecithin:cholesterol acyltransferase in cholesterol-fed rabbits: LDL metabolism and HDL metabolism are affected in a gene dose-dependent manner. J. Lipid Res. 38, 2537–2547.

Dammerman, M., Breslow, J.L., 1995. Genetic basis of lipoprotein disorders. Circulation 91, 505–512.

Geiss, H.C., Parhofer, K.G., Schwandt, P., 1999. Atorvastatin compared

with simvastatin inpatients with severe LDL hypercholesterolemia treated by regular LDL apheresis. J. Intern. Med. 245, 47–55.

Ghirlanda, G., Oradei, A., Manto, A., Lippa, S., Ussioli, L., Caputo, S., Greco, A.V., Littarru, G.P., 1993. Evidence of plasma CoQ10-lowering effects by HMG-CoA reductase: a double-blind, placebo-controlled study. J. Clin. Pharmacol. 33, 226–229.

Hiyoshi, H., Yanagimachi, M., Ito, M., Ohtsuka, I., Yoshida, I., Saeki, T., Tanaka, H., 2000. Effect of ER-27856, a novel squalene synthase inhibitor, on plasma cholesterol in rhesus monkey: comparison with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors. J. Lipid Res. 41, 1136–1144.

Hiyoshi, H., Yanagimachi, M., Ito, M., Saeki, T., Yoshida, I., Okada, T., Ikuta, H., Shinmyo, D., Tanaka, K., Kurusu, N., Tanaka, H., 2001. Squalene synthase inhibitors reduce plasma triglyceride through a low-density lipoprotein receptor-independent mechanism. Eur. J. Pharmacol. 431, 345–352.

Hobbs, H.H., Brown, M.S., Goldstein, J.L., 1992. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum. Mutat. 1, 445–446.

Hornick, C., Kita, T., Hamilton, R.L., Kane, J.P., Havel, R.J., 1983. Secretion of lipoprotein from the liver of normal and Watanabe heritable hyperlipidemic rabbits. Proc. Natl. Acad. Sci. U. S. A. 80, 6096–6100.

Hsu, I., Spinler, S.A., Johnson, N.E., 1995. Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. Ann. Pharmacother. 29, 743–759.

Ishibashi, S., Brown, M.S., Goldstein, J.L., Gerard, R.D., Hammer, R.E., Herz, J., 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J. Clin. Invest. 92, 1885–1893.

Ishibashi, S., Goldstein, J.L., Brown, M.S., Herz, J., Burns, D.K., 1994. Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J. Clin. Invest. 93, 1885–1893.

Jacob, B.G., Richter, W.O., Schwandt, P., 1993. Therapy of severe familial hypercholesterolemia by low-density lipoprotein apheresis with immunoadsorption: effects of the addition of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors to therapy. Clin. Invest. 71, 908–912.

Kita, T., Brown, M.S., Watanabe, Y., Goldstein, J.L., 1981. Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia. Proc. Natl. Acad. Sci. U. S. A. 78, 2268–2272.

Magoulas, L.M., Goldberg, I.J., Bisgaier, C.L., Serajuddin, H., Francone, O.L., Breslow, J.L., Tall, A.R., 1997. A mouse model with features of familial combined hyperlipidemia. Science 275, 391–394.

Marais, A.D., Firth, J.C., Bateman, M.E., Byrnes, P., Martens, C., Mountney, J., 1997. Atorvastatin: an effective lipid-modifying agent in familial hypercholesterolemia. Arterioscler. Thromb. Vasc. Biol. 17, 1527–1531.

Miki, T., Kori, M., Mabuchi, H., Tozawa, R., Nishimoto, T., Sugiyama, Y., Teshima, K., Yukimasa, H., 2002. Synthesis of 4,1-benzoxazepine derivatives as squalene synthase inhibitors and their inhibition of cholesterol synthesis. J. Med. Chem. 45, 4571–4580.

Nagata, Y., Zilverssmit, D.B., 1987. Blockade of intestinal lipoprotein clearance in rabbits injected with Triton WR 1339-ethyl oleate. J. Lipid Res. 28, 684–692.

Raal, F.J., Pappu, A.S., Illingworth, D.R., Pilcher, G.J., Marais, A.D., Firth, J.C., Kotzu, M.J., Heinonen, T.M., Black, D.M., 2000. Inhibition of cholesterol synthesis by atorvastatin in homozygous familial hypercholesterolaemia. Atherosclerosis 150, 421–428.

Rosenberg, S.H., 1998. Squalene synthase inhibitor. Exp. Opin. Ther. Patents 8, 521–530.

Shiomi, M., Ito, T., 1994. 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.

Shiomi, M., Ito, T., 1999. 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.

Shiomi, M., Shiraishi, T., Yata, T., Ito, T., 1994. Effect of fluvastatin sodium on secretion of very low density lipoprotein and serum cholesterol levels. In vivo study using low density lipoprotein receptor deficient Watanabe heritable hyperlipidemic rabbits. Arzneimittelforschung 44, 1154–1156.

Slack, J., 1969. Risks of ischaemic heart disease in familial hypercholesterolemic states. Lancet 2, 1380–1382.

Tsujita, Y., Kuroda, M., Shimada, Y., Tanzawa, K., Arai, M., Kaneko, I., Tanaka, M., Masuda, H., Tarumi, C., Watanabe, Y., Fujii, S., 1986. CS-514, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase: tissue-selective inhibition of sterol synthesis and hypolipidemic effect on various animal species. Biochim. Biophys. Acta 87, 50–60.

Ugawa, T., Kakuta, H., Moritani, H., Matsuda, K., Ishihara, T., Yamaguchi, M., Naganuma, S., Iizumi, Y., Shikama, H., 2000. YM-53601, a novel squalene synthase inhibitor, reduces plasma cholesterol and triglyceride levels in several animal species. Br. J. Pharmacol. 131, 63–70.

Watanabe, Y., 1980. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). Atherosclerosis 36, 261–268.

Watanabe, Y., Ito, T., Shiomi, M., 1988. Preventive effect of pravastatin sodium, a potent inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A, on coronary atherosclerosis and xanthoma in WHHL rabbits. Biochim. Biophys. Acta 960, 294–302.