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Triglyceride-lowering effect of pitvastatin in a rat model of postprandial lipemia

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Abstract

The triglyceride-lowering effect of pitavastatin, a potent 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, was investigated in a rat model of postprandial lipemia. Plasma triglyceride levels started to increase 4 h after the fat load, reached the maximum at 6 h and then gradually decreased. A single dose of pitavastatin (1 mg/kg) significantly suppressed chylomicron-triglyceride secretion into the lymph by 40% and delayed the elevation of plasma triglyceride. Pitavastatin at 1 mg/kg decreased the 6-h plasma triglyceride levels by 53% and at 0.5 mg/kg decreased the 0–12 h area under the curve (AUC) of triglyceride levels by 56%. Atorvastatin also caused decreases, but to a lesser extent. Pitavastatin, and atorvastatin to a lesser extent, reduced the activity of the intestinal microsomal triglyceride transfer protein (MTP) at 6 h. These results suggested that a single dose of pitavastatin lowered postprandial triglyceride levels in rats by decreasing chylomicron-triglyceride secretion, probably through a reduction of intestinal MTP activity and triglyceride droplet formation in the endoplasmic reticulum.

Keywords: Pitavastatin; 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitor; Model of postprandial lipemia; Lymph chylomicron; Microsomal triglyceride transfer protein

1. Introduction

The current increase in atherosclerotic mortality is ascribed at least in part to westernized food (Esreby et al., 1996). It has long been said that atherosclerosis is a postprandial phenomenon (Zilversmit, 1979). Remnants have proved to be atherogenic, and postprandial hyperlipidemia is regarded as an independent risk factor for fasting plasma hypercholesterolemia (Weintraub et al., 1996).

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are drugs that lower low-density lipoprotein-cholesterol (LDL-C) levels in plasma through the induction of the hepatic low-density lipoprotein (LDL) receptor (Maron et al., 2000). Statins also lower postprandial plasma lipids by accelerating remnant clearance (Packard, 1997; Battula et al., 2000; Burnett et al., 1998) through LDL receptors.

Pitavastatin is a potent long-acting HMG-CoA reductase inhibitor (Aoki et al., 1997; Fujino et al., 1999) which lowers plasma total cholesterol levels and reduces triglyceride levels as well (Kajinami et al., 2000). It has total cholesterol- and triglyceride-lowering effects in guinea pigs by increasing hepatic LDL receptors and by suppressing very-low-density lipoprotein (VLDL) secretion (Suzuki et al., 1999). The latter effect is characteristic of long-acting pitavastatin, but is not shared by simvastatin even at a dose 10-fold that of pitavastatin. The prolonged action of pitavastatin is caused by the contribution of the enterohepatic circulation of unmetabolized pitavastatin (Fujino et al., 1999; Kimata et al., 1998). Therefore, pitavastatin would be expected to have a prolonged action in the small intestine as well as in the liver, and the prolonged inhibition of intestinal HMG-CoA reductase may result in the suppression of chylomicron secretion analogously to liver VLDL secretion.

In the present study, we investigated the effect of pitavastatin on postprandial plasma lipids in rats, in which statins do not increase LDL receptor numbers (Fujioka et al., 1995). A single dose as low as 0.5 to 1 mg/kg of

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pitavastatin lowered postprandial triglyceride levels. Atorvastatin, for which the enterohepatic circulation and intestinal distribution of the drug are less important (Nemoto et al., 1998), also lowered the postprandial triglyceride levels, but at doses higher than those of pitavastatin. Furthermore, studies of the intestinal action of pitavastatin revealed that the drug reduced microsomal triglyceride transfer protein (MTP) activity in the jejunum and suppressed chylomicron-triglyceride secretion into the lymph.

2. Materials and methods

2.1. Test substances and chemicals

Pitavastatin was supplied by Nissan Chemical Industries (Tokyo, Japan). Atorvastatin, isolated from SORTIS (Parke-Davis, Morris Plains, NJ, USA), was extracted with methanol, concentrated, dissolved in ethyl acetate, washed with saturated saline (pH 4), and precipitated from methanol–water as atorvastatin–$3H_2O$. The test substances were suspended in a 0.5% sodium carboxymethyl-cellulose solution. Clinimeal, an oral nutrient containing 12.5 g fat (coconut oil/corn oil = 2:1) per package (89 g powder), was purchased from Eisai (Tokyo, Japan). One package was dissolved in 96 ml of water (total volume 156 ml) to make 8% fat emulsion (''clinimeal emulsion'') for the experiments. Lipid assay kits, Cholesterol E-test Wako, Triglyteride G-test Wako, Phospholipid C-test Wako, EDTA- and DEAE-cellulose were purchased from Wako (Osaka, Japan). DEAE-Sephacel was obtained from Pharmacia Biotech (Uppsala, Sweden). Bicinchoninic acid (BCA) protein assay reagent was a product of Pierce (Rockford, IL, USA). [Carboxyl-^{14}C]triolein (4.1 GBq/mmol) was purchased from DuPont/NEN (Boston, MA, USA). Deoxycholate, egg phosphatidylcholine, triolein, bovine serum albumin (fatty acid free), and butylated hydroxytoluene were obtained from Sigma-Aldrich Chemie (Steinheim, Germany).

2.2. Animals and diets

Male Wistar rats aged 8–10 weeks (Japan Laboratory Animals, Tokyo, Japan) were housed in a room maintained at 23 ± 2 °C, relative humidity at $55 \pm 15\%$, under a constant light/dark cycle (light 7:00 a.m. to 7:00 p.m.) and allowed ad libitum access to water and standard rodent chow (CE-2; Japan Clea, Tokyo, Japan).

The animal experiments were carried out with the approval of the Animal Ethics Committee in Kowa according to internationally valid guidelines.

2.3. Rat model of postprandial lipemia

Rats fasted for 18 h were administered 20 ml/kg of clinimeal emulsion and then allowed free access to drinking water, but not to the chow. Blood samples (collected in tubes containing 1 mg/ml of EDTA, pH 7.4) were taken from the jugular vein, before ($t = 0$) and 2, 4, 6, 8, 10, 12, 24 h after the fat load. The plasma was separated and measured for triglyceride levels with the diagnostic kit.

To evaluate the effect of statins, pitavastatin (0.5, 1 and 2 mg/kg), atorvastatin (1, 2 and 4 mg/kg) or the vehicle alone (control) was administered immediately before the fat load. The doses were determined according to the ED_{50} of liver sterol synthesis: 0.13 mg/kg for pitavastatin (Aoki et al., 1997) and 0.24 mg/kg for atorvastatin (data not shown). The effect was evaluated by measuring triglyceride levels at each point in time, especially at 6 h, and the area under the curve (AUC) for triglyceride levels above the baseline from 0 to 12 h was calculated.

2.4. Lipoprotein fractionation

To examine the characteristics of the experimental model, the 6-h plasma in the control was pooled and ultracentrifuged at 17,600 rpm for 30 min at 16 °C (SW40Ti, Beckman, Fullerton, CA, USA) to separate the chylomicron fraction and then at 100,000 rpm for 2.5 h to obtain VLDL ($d < 1.006$) (TL100.4, Beckman). IDL ($1.006 < d < 1.019$), LDL ($1.019 < d < 1.063$) and HDL ($1.063 < d < 1.210$) were obtained by further sequential ultracentrifugation. Chylomicron and its remnant were separated according to the method of Felts (1987). Briefly, a portion (200 μl) of the chylomicron fraction was loaded on a DEAE-Sephacel column (1.8 ml of wet gel) equilibrated with 0.15 M NaCl, 0.02 M Tris, pH 8.6. Chylomicron was obtained as a nonadsorbed fraction, and the chylomicron remnant was eluted with a NaCl gradient 0.15 to 1 M in 0.02 M Tris. Chylomicron and the chylomicron remnant were concentrated by ultracentrifugation. Each lipoprotein was measured for triglyceride content with the relevant diagnostic kit.

2.5. Analysis of lymph chylomicron

The rats were fasted for 17–20 h (from 4 p.m. to 9–12 a.m.). Under anesthesia (50 mg/kg), a cannula was inserted in the thoracic lymph duct and the stomach and then the rats were placed in Bollman cages (Miura et al., 1993). Saline was infused into the stomach overnight to stabilize lymph flow. Pitavastatin (1 mg/kg) or the vehicle alone (control) and then 20 ml/kg clinimeal emulsion were injected into the stomach, and the lymph was collected every 1 h up to 8 h. Chylomicron fractions ($d < 1.006$ g/ml) were obtained by layering the lymph underneath 0.15 M NaCl, followed by centrifugation at 100,000 rpm for 2.5 h (TL100.4, Beckman). The chylomicron-containing top layer was isolated and measured for total cholesterol, triglyceride and phospholipid with diagnostic kits. Total protein contents were determined with the BCA assay reagent.

The amounts of chylomicron-lipids and chylomicron-protein secreted were calculated by multiplying by the lymph volume. Chylomicron-triglyceride secretion rate

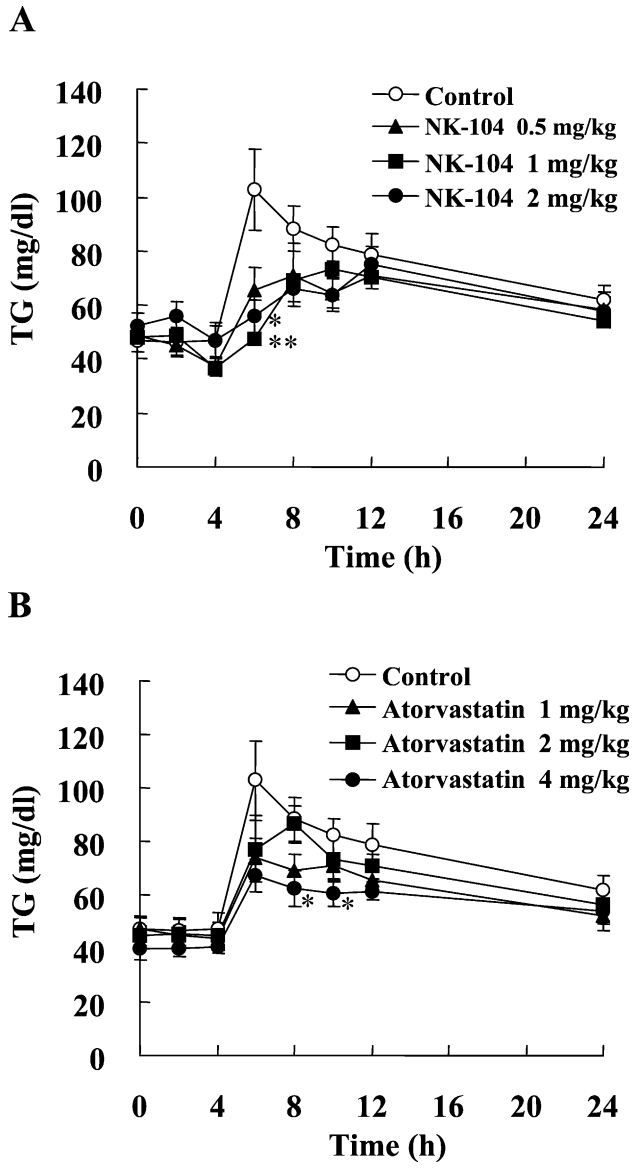

Fig. 1. Effect of pitavastatin (A) and atorvastatin (B) on triglyceride levels. Statins were orally administered orally immediately before loading fasted rat with "clinimeal emulsion". Values are the means ± S.E. ($n = 10$). * $P < 0.05$, ** $P < 0.05$ vs. control (Dunnett).

was determined by linear regression from the plots of the cumulative amount of chylomicron-triglyceride secreted (1 to 8 h) in each animal.

Furthermore, the lymph samples collected from 3 to 5 h were combined, ultracentrifuged to separate chylomicron and analyzed for chylomicron-lipid composition.

2.6. Measurement of intestinal MTP activity

The jejunum was isolated 6 h after the statin gavage and the fat (clinimeal emulsion) load was given to fasted rats. MTP activity was measured as described (Wetterau and Zilversmit, 1986). Briefly, about 3 g of the jejunum was homogenized in a Downce homogenizer with 3 volumes of 50 mM Tris, 250 mM sucrose, 1 mM EDTA, 0.02% NaN_3, pH 7.4. The homogenate was fractionated by sequential ultracentrifugation; the microsome fraction was collected as the 700 × g supernatant, the 12,000 × g supernatant and the 105,000 × g precipitate.

The microsome suspension (adjusted to 3 mg protein/ml, 50 mM Tris, 50 mM KCl, 5 mM $MgCl_2$, pH 7.4) was mixed with 0.1 ml of 0.54% deoxycholeate (pH 7.4), allowed to stand for 30 min at 4 °C, and ultracentrifuged to obtain microsomal lumenal proteins as supernatant. This fraction was dialyzed against an assay buffer (15 mM Tris–HCl, 35 mM NaCl, 1 mM EDTA, 0.02% NaN_3, pH 7.4), measured for protein content and stored at −20 °C.

Triglyceride transfer activity of the stored samples was measured using donor vesicles (composed of 35 nmol egg phosphatidylcholine, 0.175 nmol triolein, 185 Bq [^{14}C]triolein and 0.1% butylated hydroxytoluene) and acceptor vesicles (composed of 175 nmol egg phosphatidylcholine, 0.875 nmol triolein, 8.8 nmol cardiolipin and 0.1% butylated hydroxytoluene). These vesicles, bovine serum albumin (5 mg) and the microsomal lumenal proteins (50 μg) were mixed in the assay buffer (total 1 ml) and allowed to react for 1 h at 37 °C. The resultant mixture was augmented with 0.5 ml DEAE-cellulose suspension (wet gel/aqueous layer = 1/1 by volume) and shaken vigorously for 1 min to adsorb acceptor vesicles. A portion (0.4 ml) of the supernatant (8000 × g, 3 min) containing only donor vesicles was measured for radioactivity. Triglyceride transfer (%) was calculated from the radioactivity by using the following equation: triglyceride transfer (%) = 100 × [1 − sample/blank], where blank represents the radioactivity without the MTP source. These values were then calculated into the absolute triglyceride transfer activity per weight microsomal protein.

2.7. Statistical analysis

Values are expressed as means ± S.E. Statistical analyses were carried out with Dunnett's multiple comparison test

Table 1
Lipoprotein–triglyceride levels in fat-loaded rats

Lipoprotein	Triglyceride concentration (mg/dl)	
	Normal	6 h after the fat load
Chylomicron	5 ± 1	29 ± 3[a]
Chylomicron		3 ± 2
Chylomicron remnant		16 ± 4
VLDL	21 ± 1	38 ± 5[b]
IDL	6 ± 1	8 ± 1
LDL	2 ± 0	4 ± 0
HDL	1 ± 0	2 ± 0

The 6-h plasma was ultracentrifuged and measured for lipoprotein–triglyceride levels. The chylomicron fraction was further separated into chylomicron and the chylomicron remnant with DEAE-Sephacel. Values are means ± S.E. ($n = 5$). Normal: normal rats without the fat load.

[a] $P < 0.01$ vs. normal (Student's t-test).

[b] $P < 0.05$ vs. normal (Student's t-test).

Table 2
Effect of pitavastatin and atorvastatin on postprandial plasma triglyceride levels in rats

	Dose (mg/kg)	Plasma triglyceride levels	
		6 h (mg/dl)	ΔAUC (mg h/dl)
Control		103 ± 15	318 ± 53
Pitavastatin	0.5	65 ± 9	140 ± 30[a]
	1	48 ± 3[b]	142 ± 29[a]
	2	56 ± 6[a]	108 ± 21[b]
Atorvastatin	1	74 ± 7	164 ± 28[a]
	2	77 ± 13	243 ± 38
	4	67 ± 6	179 ± 34[a]

From Fig. 1, the 6-h plasma triglyceride levels and the 0–12 h AUC of plasma triglyceride levels above the initial level (ΔAUC) are summarized. Values are means ± S.E. ($n = 10$).

[a] $P < 0.05$ vs. control (Dunnett).

[b] $P < 0.01$ vs. control (Dunnett).

preceded by Bartlett analysis of variance. Differences of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Analysis of elevated triglyceride

In the rat model of postprandial lipemia, mean plasma triglyceride levels started to increase at 4 h after the fat (clinimeal emulsion) load, reached the maximum at 6 h, and then gradually decreased (Fig. 1).

Triglyceride levels in each lipoprotein fraction in the 6-h plasma are shown in Table 1. The fat load significantly increased chylomicron-triglyceride and VLDL-triglyceride. In the chylomicron fraction, the increase in triglyceride was mainly due to that in the chylomicron remnant. Therefore, in our model of postprandial lipemia, the triglyceride increase in plasma was attributed to that in the chylomicron remnant and the VLDL.

3.2. Effect of pitavastatin and atorvastatin on postplandial triglyceride levels

The time course of plasma triglyceride levels and the effect of pitavastatin or atorvastatin given just before the fat load are shown in Fig. 1. Pitavastatin at 1 mg/kg significantly lowered the 6-h plasma triglyceride levels by 53% (from 103 to 48 mg/dl). Atorvastatin at 4 mg/kg had a similar, but smaller, effect and significantly lowered the 8- and 10-h plasma triglyceride levels by 30% (from 88 to 62 mg/dl) and 26% (from 82 to 61 mg/dl), respectively.

The 6-h plasma triglyceride levels (the maximum in control) and the 0–12-h AUC of plasma triglyceride levels above the initial level (Δtriglyceride AUC) are shown in Table 2. Pitavastatin at 0.5 mg/kg or more significantly lowered the AUC by 55–66%; at 1 mg/kg or more, it

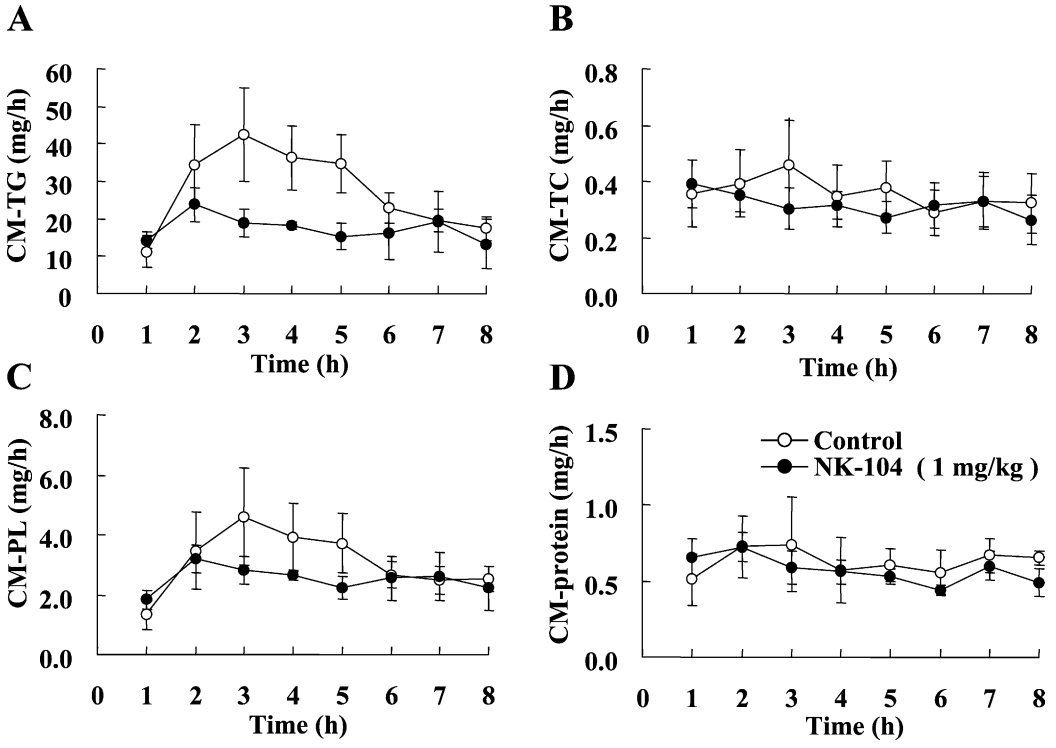

Fig. 2. Effect of pitavastatin on chylomicron secretion into the lymph. After fat loading of the rats, the lymph was collected through the thoracic lymph duct every 1 h up to 8 h. Lymph chylomicron was separated by ultracentrifugation and measured for (A) triglyceride, (B) total cholesterol, (C) phospholipid and (D) protein concentration. The secreted amounts of these components were determined by multiplying by the lymph volume. Values are means ± S.E. ($n = 4$).

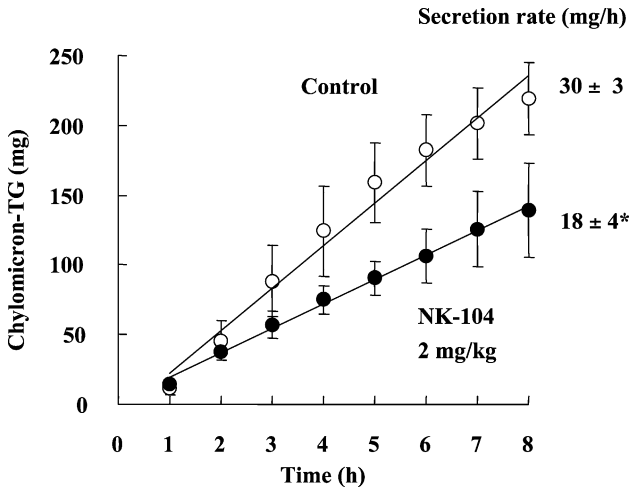

Fig. 3. Effect of pitavastatin on the rate of chylomicron-triglyceride secretion rate into the lymph. From the data shown in Fig. 2, cummulative chylomicron-triglyceride secreted into the lymph was plotted. The chylomicron-triglyceride secretion rate was determined by linear regression from the plots for each animal and is shown as the means ± S.E. ($n=4$). * $P<0.05$ (Student's t-test). Each point in the figure represents the means ± S.E. and the lines represent the linear regression of the mean values.

decreased the 6-h triglyceride levels by 46% to 53%. Atorvastatin at 1 and 4 mg/kg significantly lowered the AUC by 48% and 44%, respectively.

3.3. Effect on lymph chylomicron

The time course of chylomicron-lipid and -protein secretion into the lymph is shown in Fig. 2. Pitavastatin (1 mg/kg) decreased the 2- to 5-h secretion of chylomicron-triglyceride and chylomicron-phospholipid very similarly, but not significantly; chylomicron-total cholesterol and chylomicron-protein secretion was not affected.

The cumulative amount and the rate of secretion of chylomicron-triglyceride into the lymph are shown in Fig. 3. Pitavastatin reduced the chylomicron-triglyceride secretion rate significantly by 40% (from 30 to 18 mg/h).

Triglyceride/protein, total cholesterol/protein and phospholipid/protein ratios in the 3- to 5-h lymph chylomicron (combined) are shown in Table 3. Pitavastatin decreased the triglyceride and phospholipid ratios by 35% (from 48 to 31 mg/mg protein) and 25% (from 6.1 to 4.6 mg/mg protein), respectively; the total cholesterol ratio showed little change.

Table 3
Effect of pitavastatin on lipid composition of lymph chylomicron

	Control	Pitavastatin
	Ratios	
Triglyceride/protein	48 ± 9	31 ± 4
Total cholesterol/protein	0.61 ± 0.03	0.69 ± 0.05
Phospholipid/protein	6.1 ± 0.8	4.6 ± 0.4

The lymph obtained at 3, 4 and 5 h shown in Fig. 2 was combined. The chylomicron fraction was separated by ultracentrifugation and measured for lipid and protein contents. Weight ratios of lipids per protein are shown in the table. Values are means ± S.E. ($n=4$).

Table 4
Effect of statins on intestinal MTP activity

	MTP (triglyceride transfer) activity (nmol/mg protein/h)		
	Control	Pitavastatin, 2 mg/kg	Normal
[A] Experiment 1	0.116 ± 0.005	0.092 ± 0.006[a] (−21%)	0.109 ± 0.005
	Control	Pitavastatin, 2 mg/kg	Atorvastatin, 4 mg/kg
[B] Experiment 2	0.122 ± 0.010	0.082 ± 0.006 (−33%)	0.099 ± 0.019 (−19%)

At 6 h after the statin gavage and the fat load, the jejunum was isolated to separate microsomes, and the microsomal lumenal protein was obtained by deoxycholate treatment. With 50 μg of the lumenal protein as MTP source, [^{14}C]triolein transfer (%) from donor to acceptor vesicles for 1 h was determined, and the triglyceride transfer activity per weight microsomal protein (nmol/mg protein/h) was calculated. Values are means ± S.E. ($n=6$). Normal: normal rats without the fat load. Values in parenthesis represent percent change from control.

[a] $P<0.05$ vs. control ([A]Student's t-test, [B] Dunnett).

3.4. Effect on intestinal MTP activity

Intestinal MTP activity 6 h after a single dose of statins and the fat load is shown in Table 4. The triglyceride transfer (%) from donor to acceptor vesicles in the control, pitavastatin and normal groups was 26.6 ± 0.5%, 24.6 ± 0.7% and 25.8 ± 0.6% (Table 4A), and in the control, pitavastatin and atotrvastatin groups, it was 20.5 ± 1.8%, 16.1 ± 1.3% and 15.4 ± 2.5% (Table 4B), respectively. The respective microsomal lumenal/total protein ratios were 0.124 ± 0.004, 0.107 ± 0.004 and 0.121 ± 0.004 (Table 4A), and 0.175 ± 0.016, 0.148 ± 0.009 and 0.180 ± 0.011 (Table 4B), respectively. The calculated values of MTP (triglyceride transfer) activity per microsomal protein (nmol/mg protein/h) for pitavastatin ([A] 0.092 ± 0.006 and [B] 0.082 ± 0.006) were [A] 21% and [B] 33% lower than those of control ([A] 0.116 ± 0.005 and [B] 0.122 ± 0.010), respectively. Atorvastatin ([B] 0.099 ± 0.019) also lowered MTP activity, but to a lesser extent (19% from control). At 2 h, pitavastatin did not affect intestinal MTP activity (data not shown).

4. Discussion

We examined the effect of pitavastatin on postprandial triglyceride levels to determine whether pitavastatin, during its enterohepatic circulation, suppresses chylomicron secretion through prolonged inhibition of intestinal HMG-CoA reductase (Kimata et al., 1998).

In the rat model of postprandial lipemia established by loading with a clinimeal emulsion, the mean triglyceride levels started to increase 4 h after the fat load and reached a maximum at 6 h, which was mainly attributed to an increase

in chylomicron remnant-triglyceride and VLDL-triglyceride (Fig. 1, Table 1). At 1 to 2 h, an abundance of chylomicron-triglyceride in the lymph would have passed into the blood, but the plasma triglyceride levels did not increase until 4 h. This suggested that widespread occupation or saturation of liver-binding sites with remnants (Mahley and Ji, 1999) may be a requisite for plasma triglyceride increases.

Pitavastatin (1 mg/kg) significantly decreased the chylomicron-triglyceride secretion rate into the lymph by 40% (Fig. 3) which, in turn, decreased the rate of chylomicron-triglyceride transfer to the blood to delay the saturation of remnant-binding sites. Thus, pitavastatin at 1 mg/kg was assumed to have blunted the elevation of postprandial triglyceride and to have lowered the 6-h plasma triglyceride levels (Fig. 1). This intestinal action was also observed for atorvastatin, but to a lesser extent, judging from the slope of the 4- to 6-h plasma triglyceride elevation. Atorvastatin at 4 mg/kg significantly reduced the 10- and 12-h triglyceride levels, which could have been caused by a different mechanism such as enhanced clearance attributable to reduced VLDL competition derived from suppressed VLDL secretion.

Prolonged inhibition of liver sterol synthesis by pitavastatin reduces VLDL secretion because of decreased cholesterol ester supply (Suzuki et al., 1999). Decrease cholesterol ester supply reduces primordial apoB lipoprotein formed in the first step of VLDL production which, in turn, limits triglyceride load in the second step (Olofsson et al., 1999) and VLDL-triglyceride secretion. In the present study, however, the reduction of chylomicron secretion by pitavastatin was observed only for triglyceride and phospholipid, but not for total cholesterol and proteins. In rats, statins do not affect plasma cholesterol levels, but at high doses, they lower triglyceride levels (Krause and Newton, 1995). Therefore, statins may affect the second step of apoB lipoprotein production independently of the first step, particularly in rats. Triglyceride droplets fused with primordial apoB lipoproteins in the second step are formed from diacylglycerol and acyl-CoA mediated by diacylglycerol acyltransferase in the presence of MTP at the smooth endoplasmic reticulum (Abo-Hashema et al., 1999; Wang et al., 1999). We measured intestinal MTP activity. MTP, a microsomal lumenal protein, is a heterodimer molecule composed of a large subunit with a catalytic site and protein disulfide isomerase, both of which are necessary for MTP activity (Wetterau et al., 1991). Statins may affect the transcription of the MTP large subunit protein, which is suppressed by the sterol regulatory element-binding protein (SREBP) (Sato et al., 1999). MTP protein levels would not change rapidly, however, because of the slow turnover of the protein ($t_{1/2}$ = 4.4 days in HepG2 cells: Lin et al., 1995). Interestingly, pitavastatin decreased intestinal MTP activity at 6 h by 21% (Table 4A), as confirmed in the second experiment (Table 4B). Atorvastatin also lowered MTP activity, but to a lesser extent (Table 4B). The reason for the decrease in MTP activity is not known. Statins may affect the retrograde transport (Johnson and Haijh, 2000) of microsomal lumenal proteins, especially that of MTP, and their degradation by the cytosol proteasome (Sommer and Wolf, 1997). Thus, the decrease in MTP activity was either larger than that of the microsomal lumenal/total protein ratio or independent of the latter. Alternatively, statins may affect protein disulfide isomerase phosphorylation with ATP (Guthapfel et al., 1996; Quemeneur et al., 1994) and alter MTP activity. The reduction of intestinal MTP activity by pitavastatin may have contributed to the decrease in the secretion of chylomicron-triglyceride and chylomicron-phospholipid (Figs. 2 and 3), through the reduction of triglyceride droplet formation at the smooth endoplasmic reticulum. Since the reduction of MTP activity was observed at 6 h, however, but not at 2 h, the possibility that statins affect triglyceride and phospholipid synthesis and/or transfer independently of MTP cannot be ruled out. Chylomicron comprises spherical lipid particles formed mainly of diet-derived newly synthesized triglyceride (85–92%) and endogenously preformed phospholipid (6–12%), and the amount of triglyceride and phospholipid represents chylomicron volume and surface area, respectively (Hussain, 2000). It is notable that the secretion of chylomicron-triglyceride and chylomicron-phospholipid into the lymph showed a very similar time course and was similarly suppressed by pitavastatin (Fig. 2). These results imply that triglyceride droplets fused with primordial apoB in the second step of VLDL production may be formed at a constant phospholipid/triglyceride ratio at the smooth endoplasmic reticulum and that pitavastatin may have suppressed triglyceride droplet formation through MTP or independently of MTP.

In conclusion, in our rat model of postprandial lipemia, a single dose of pitavastatin (four to eight times the ED_{50} of liver sterol synthesis) or atorvastatin (17 times the ED_{50}) suppressed the increase in plasma triglyceride. The effect of pitavastatin was due to its the intestinal action: suppression of the secretion of chylomicron-triglyceride and chylomicron-phospholipid into the lymph, but not of chylomicron-total cholesterol or of chylomicron-protein. The decrease in intestinal MTP activity induced by pitavastatin may have contributed to the reduction of chylomicron-triglyceride and chylomicron-phopholipid secretion through the suppression of triglyceride droplet formation in the endoplasmic reticulum.

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