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A gastrointestinal lipase inhibitor reduces progression of atherosclerosis in mice fed a western-type diet

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Abstract

To investigate whether gastrointestinal lipase inhibition reduces the progression of a western-type diet induced atherosclerosis, male apolipoprotein-E knockout (apoE KO) mice were administered orlistat ((*S*)-1-[[(*S*, 2*S*, 3*S*)-3-hexyl-4-oxo-2-oxetanyl] methyl]dodecyl-(*S*)-2-formamido-4-methylvalerate) mixed with a western-type diet for 8 weeks. Orlistat significantly reduced plasma triglyceride levels, but not total cholesterol levels, at 4 and 8 weeks of treatment. Increase in plasma triglyceride levels after oral olive oil loading in the mice fed a western-type diet was significantly suppressed in the orlistat treated group at 4 weeks of treatment. After 8 weeks treatment, atherosclerotic lesion area in the aorta of the orlistat treated group was significantly smaller than that of the control group. These results suggest that gastrointestinal lipase inhibition reduces the progression of atherosclerosis through a triglyceride-lowering effect, via inhibition of fat absorption.

Keywords: Western-type diet; Orlistat; Fat absorption; Atherosclerosis

1. Introduction

Patients with atherogenic dyslipidemia are at high risk for coronary heart disease (Gotto, 2002). Guidelines from the National Cholesterol Education Program recommend reduction of low density lipoprotein cholesterol as the primary goal of cardiovascular risk reduction therapy (Expert panel on detection, and evaluation, and treatment of high blood cholesterol in adults, 2001). According to the guideline, statins are widely used for low density lipoprotein cholesterol-lowering therapy. However, recent meta-analyses of prospective studies indicate that increased triglyceride level is also an independent risk factor for coronary heart disease (Cullen, 2000). Furthermore, abnormal postprandial accumulations of triglyceride-rich lipoproteins have been shown to be linked to coronary heart disease regardless of fasting triglyceride levels (Patsch et al., 1992; Weintraub et al., 1996). Fibrates are often used for triglyceride-lowering therapy; however, they have a partial effect on postprandial increase in triglyceride levels (Simpson et al., 1990; Wilmink et al., 2001).

Orlistat ((*S*)-1-[[(*S*, 2S, 3S)-3-hexyl-4-oxo-2-oxetanyl] methyl]dodecyl-(*S*)-2-formamido-4-methylvalerate) is an inhibitor of pancreatic and other lipases and when orally administered, effects are restricted to the gastrointestinal tract (McNeely and Benfield, 1998). As inhibition of gastrointestinal lipases leads to a reduction of fat absorption, orlistat is used as an anti-obesity drug (Ballinger and Peikin, 2002). In an animal study, orlistat also inhibited absorption of dietary ingested fats and reduced plasma triglyceride levels (Hogan et al., 1987). In patients with hyperlipidemia, orlistat has been shown to reduce postprandial plasma triglyceride levels (Reitsma et al., 1994). Therefore, orlistat may reduce the progression of atherosclerotic lesions as a result of the triglyceride-lowering effect via inhibition of fat absorption.

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Apolipoprotein E knockout (apoE KO) mice are widely used as an animal model of atherosclerosis. When fed a western-type diet, these mice have severe hyperlipidemia and atherosclerosis (Plump et al., 1992; Zhang et al., 1994). To elucidate the influence of gastrointestinal lipase inhibition on the development of atherosclerosis, we examined the effects of orlistat on fat absorption, plasma lipid profiles and atherosclerotic lesion area in apoE KO mice fed a western-type diet.

2. Materials and methods

2.1. Materials

Powdered orlistat was extracted from the commercial product (Xenical®, Hoffmann-La Roche, Basel, Switzerland) and purified at Fujisawa Pharmaceutical (Osaka, Japan). Sodium pentobarbital (Nembutal®) was purchased from Dainippon Pharmaceutical (Osaka, Japan). Triglyceride E Test Wako, Cholesterol E Test Wako and oil red O were purchased from Wako Pure Chemical (Osaka, Japan). All other reagents were purchased from Nacalai Tesque (Osaka, Japan).

2.2. Animals and diets

Homozygous apoE KO mice were bred from breeding pairs obtained from Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed in a temperature-controlled facility with a 12-h light/dark cycle and allowed ad libitum access to water. Male mice were fed either a western-type diet, containing 21% w/w fat and 0.15% w/w cholesterol, or a chow diet, containing 5% w/w fat, prepared as a control diet from 6 weeks of age (Plump et al., 1992). Both diet types were purchased from Oriental Yeast (Osaka, Japan). At 7 weeks of age, mice fed a western-type diet were divided into two diet groups: the western-type diet alone (control group) or supplemented with orlistat (orlistat group). Body weight and average food consumption for 2 or 3 days was monitored weekly. Orlistat was mixed with the western-type diet at a concentration of 150–204 ppm to deliver a dosage of approximately 20 mg/kg/day, corresponding to the dose that reduced fat absorption in rodents (Hogan et al., 1987). All animal experimental procedures were performed according to guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical.

2.3. Plasma lipid level, fecal fat excretion and atherosclerotic lesions

2.3.1. Plasma lipid measurement

Blood was collected from the retroorbital venous plexus before and at 4 and 8 weeks of treatment. Plasma total cholesterol and triglyceride levels were enzymatically measured with Cholesterol E Test Wako and Triglyceride E Test Wako, respectively.

2.3.2. Determination of fat in feces

At the 5th week of treatment, the feces were collected for 3 days and pulverized with a mill. The amount of fat in feces was analyzed using the method of van de Kamer et al. (1949) and excretion of fecal fat was expressed as a percentage of dietary ingested fat.

2.3.3. Evaluation of atherosclerotic lesions

At the 8th week of treatment, mice were euthanized for an evaluation of atherosclerotic lesions. After mice were exsanguinated under sodium pentobarbital anesthesia, the thoracic aorta was perfusion-fixed with 4% paraformaldehyde (pH 7.4) and opened longitudinally and pinned out on a black cork board. Fatty streak lesions were stained with oil red O. En face images of the aorta were taken with a digital camera (Nikon, Tokyo, Japan). Quantification of the images was performed using Mac Scope software (Mitani, Fukui, Japan). Lesion areas were calculated by dividing oil red O-stained area by total area.

2.4. Oral fat-loading test

To assess postprandial increase in triglyceride-rich lipoproteins, oral fat-loading tests were performed at the fourth week of treatment. Olive oil was chosen for the loading tests because of its safety and adequate fluidity. Mice fasted for 16 h were orally administered with 10 ml/kg of olive oil emulsion, containing 30% olive oil, 5% Tween-20 and 65% methylcellulose solution (0.5% w/v). Blood samples were collected before (0 h), plus 2 and 4 h after oral fat-loading. Plasma was separated and triglyceride level was measured, since changes in triglyceride levels after oral fat-loading correlated with changes in remnant-like particles derived from triglyceride-rich lipoproteins (Tanaka et al., 1998). Increase in plasma triglyceride levels after oral fat-loading and the area under the curve (AUC 0–4 h) were calculated.

2.5. Statistical analysis

Data are expressed as mean±S.E.M. Statistical analysis of mean differences was performed with Student's *t*-test. Values of $P<0.05$ were regarded as statistically significant.

3. Results

3.1. Effect of orlistat on hyperlipidemia and atherosclerosis

3.1.1. Body weight and food consumption

The western-type diet-fed control mice had an increase in the mean daily caloric intake of fat (4.1 kcal/mouse/day), but a significant decrease in daily food consumption (Table 1),

Table 1
Body weight and food consumption in apoE KO mice

	N	Body weight (g)			Food consumption (g/mouse/day)
		Initial	Final	Gain	
Chow diet	9	22.1±0.5	31.0±0.4	8.5±0.3	5.81±0.49
Western-type diet					
Control	9	21.3±0.3	28.9±0.5	7.6±0.6	2.60±0.09[a]
Orlistat	9	21.3±0.5	29.9±0.6	8.6±0.4	2.98±0.03[b]

Mice were fed a chow diet, a western-type diet alone (Control) or supplemented with 20 mg/kg/day orlistat for 8 weeks. Data are mean±S.E.M.
[a] $P<0.01$ compared with chow diet.
[b] $P<0.01$ compared with western-type diet control.

compared to the chow diet-fed mice (2.7 kcal/mouse/day). The food consumption of the orlistat group was significantly higher than that of the control group. There was no significant difference between the chow diet group and the western-type diet control group in body weight gain. In the western-type diet-fed mice, body weight gain was not affected by orlistat treatment.

3.1.2. Plasma lipid level

Before and at 4 and 8 weeks of treatment, both plasma triglyceride and total cholesterol levels in the western-type diet-fed control mice were significantly higher than in the chow diet-fed mice (Fig. 1). In the mice fed a western-type diet, orlistat significantly lowered plasma triglyceride levels to those in the mice fed a chow diet (Fig. 1A). However, orlistat had no significant effect on plasma total cholesterol levels in the mice fed a western-type diet (Fig. 2B).

3.1.3. Fecal fat excretion

At the fifth week of treatment, excretion of fecal fat in the chow diet-fed mice and the western-type diet-fed control mice were 3% and 4% of ingested fat, respectively. Orlistat markedly increased excretion of fecal fat (40% of ingested fat) in the mice fed a western-type diet.

3.1.4. Atherosclerosis

Fig. 2A through C show representative en face surface of the thoracic aortas. In the western-type diet-fed control mice, atherosclerotic lesions stained with oil red O were

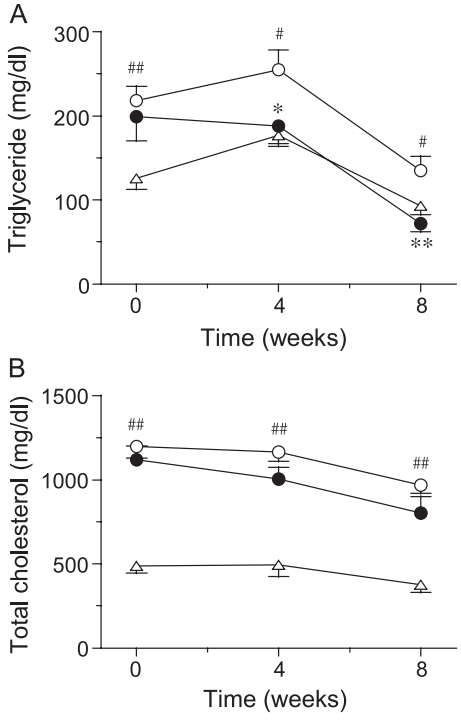

Fig. 1. Effect of orlistat on plasma triglyceride (A) and total cholesterol levels (B) in apoE KO mice. Treatment with orlistat (20 mg/kg/day) as a dietary mixture was started at 6 weeks of age. Open triangle: chow diet (n=8), open circle: western-type diet control (n=8), closed circle: western-type diet supplemented with orlistat (n=9). Values are mean±S.E.M.

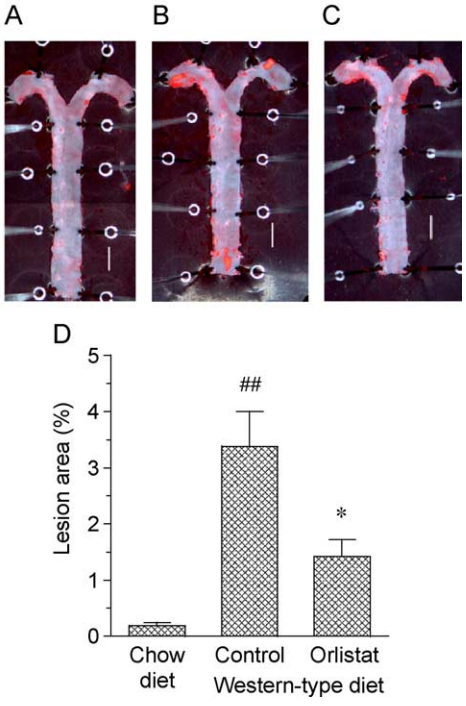

Fig. 2. Effect of orlistat on atherosclerotic lesions in apoE KO mice. Fatty streak lesions were stained with oil red O after the eighth week of treatment. Photographs show representative en face thoracic aorta preparations of mice fed a chow diet (A), a control western-type diet (B), and a western-type diet with orlistat (C). The quantitative analysis of en face lesion area (D) shows that orlistat significantly reduced the lesion area in the mice fed a western-type diet. Values are mean±S.E.M. n=8–9, $^{\#\#}P<0.01$ compared with chow diet, $^{*}P<0.05$ compared with western-type diet control.

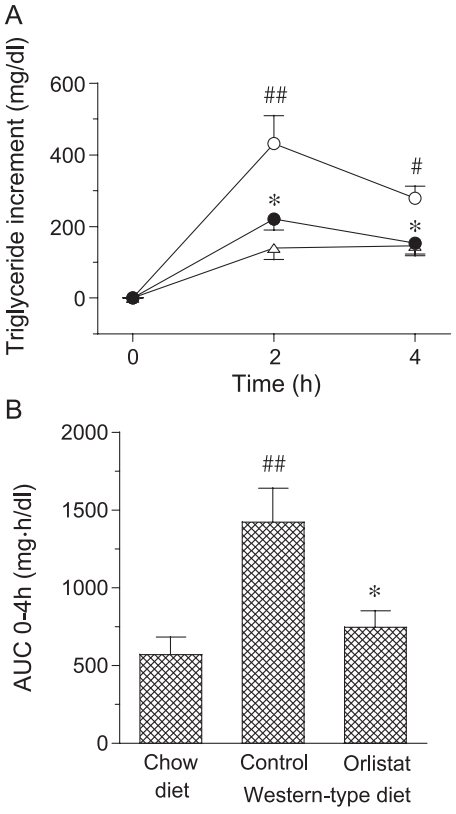

Fig. 3. Effect of orlistat on plasma triglyceride increase (A) and the AUC 0–4 h (B) after oral fat-loading in apoE KO mice. Mice fed a chow diet (open triangle; n=7), a control western-type diet (open circle; n=7) or a western-type diet with orlistat (closed circle; n=7) were fasted for 16 h and administered 30% olive oil emulsion. AUC of triglyceride increase in the western-type diet-fed mice (B) was significantly suppressed by orlistat treatment. Basal triglyceride levels in mice fed the chow diet, the western-type diet alone (Control) and supplemented with orlistat were 140±16, 135±13 and 123±18 mg/dl, respectively. Values are mean±S.E.M. $^{\#\#}P<0.01$ and $^{\#}P<0.05$ compared with chow diet, $^{*}P<0.05$ compared with western-type diet control.

found in the aortic root, the lesser curvature of the aortic arch and the principal branches of the thoracic aorta (Fig. 2B). The lesion area of the western-type diet control group was significantly larger than that of the chow diet group ($P<0.01$) (Fig. 2D). Orlistat significantly reduced lesion area by 58% ($P<0.05$) in the mice fed a western-type diet.

3.2. Effect of orlistat on increase in plasma triglyceride levels after oral fat-loading

Plasma triglyceride levels were significantly higher with a peak at 2 h after oral fat-loading in the western-type diet-fed control mice, compared to the chow diet-fed mice (Fig. 3A). AUC of triglyceride increase in the western-type diet control group was 2.5-fold higher than that of the chow diet group (Fig. 3B). In the orlistat-treated mice, increase in triglyceride levels after oral fat loading was significantly suppressed and the triglyceride curve was similar to that of the chow diet-fed mice.

4. Discussion

The present study demonstrated that orlistat significantly reduced the progression of atherosclerotic lesions, concomitantly with lowering of plasma triglyceride levels, but not total cholesterol levels, in apoE KO mice fed a western-type diet. In addition, orlistat reduced plasma triglyceride increase after oral fat-loading. These results suggest that gastrointestinal lipase inhibition reduces the progression of atherosclerosis through a triglyceride-lowering effect via inhibition of intestinal triglyceride absorption.

Orlistat inhibits intestinal fat absorption secondary to inhibition of gastrointestinal lipases, the central enzyme in triglyceride hydrolysis in the gastrointestinal tract (McNeely and Benfield, 1998), and is used as an anti-obesity drug (Ballinger and Peikin, 2002). In our study, we used orlistat at a dosage of approximately 20 mg/kg/day, which increased fecal fat excretion with a 10-fold increase compared to control. Thus, it was confirmed that this dose of orlistat was sufficient for the inhibition of fat absorption in the apoE KO mice.

Although orlistat showed significant increases in fecal fat excretion in this study, it had no effect on body weight change in apoE KO mice fed a western-type diet. In obese patients (McNeely and Benfield, 1998) and high fat diet-induced obese rats (Hogan et al., 1987; Ackroff and Sclafani, 1996), orlistat reduced body weights concomitantly with an increase in fecal fat excretion. The discrepancies between our results and previous studies might be accounted for both slight increase in food consumption in orlistat group and the characteristics of adipogenesis in apoE KO mice. An increase in food intake was also observed in the rats receiving orlistat (Hogan et al., 1987). ApoE has been shown to be involved in receptor-mediated uptake of very low-density lipoprotein into adipocytes (Chiba et al., 2003). Therefore, adipogenesis in response to fat absorbed from the diet is likely to be minimal in apoE KO mice. In fact, body weight gain in the mice fed a western-type diet was almost equivalent to that of the chow diet-fed mice. This observation suggests that body weight in apoE KO mice was hardly affected by the amount of ingested dietary fat.

In apoE KO mice, orlistat lowered plasma triglyceride levels but not total cholesterol levels. This is consistent with the findings in high fat diet induced obese rats (Hogan et al., 1987). The differences in the effects of orlistat on triglyceride and total cholesterol levels might be explained by the finding of Fernandez and Borgström (1988). They showed that orlistat reduced recovery of lymphatic radioactivity from radio-labeled triolein, but not radio-labeled cholesterol, infused intraduodenally in rats, suggesting that orlistat inhibited triglyceride absorption, but not cholesterol absorption.

ApoE KO mice are used as a model to develop atherosclerotic lesions similar to those in humans (Nakashima et al., 1994). In our results, orlistat reduced the surface lesion areas in the thoracic aorta, but we did not

perform pathological analysis of atheroma except for the en face lipid-staining. Tangirala et al. (1995) demonstrated that there was a significant correlation between the surface areas of lipid-stained lesion in the entire aorta and the cross-sectional areas of neointimal hyperplasia at the aortic root in apoE KO mice. Furthermore, Tordjman et al. (2001) demonstrated that macrophages were prominent throughout the lipid-filled neointima of the aortic root in apoE KO mice fed a western-type diet. Taken together, orlistat should reduce neointimal macrophage-derived foam cells and subsequently decreases progression of atherosclerotic lesions. Further studies might be needed to clarify these mechanisms.

The benefit of cholesterol-lowering therapy in the prevention of atherosclerosis is well established. Davis et al. (2001) demonstrated that cholesterol absorption inhibitor, ezetimibe, almost completely reduced atherosclerotic lesions concomitantly with a lowering of both total cholesterol and triglyceride levels in apoE KO mice fed a western-type diet. Other study using hypercholesterolemic rats demonstrated that a lipoprotein lipase activator, ibrolipim, suppressed the development of atherosclerosis concomitantly with the triglyceride-lowering and the high density lipoprotein cholesterol elevation (Tsutsumi et al., 1993). Our results showed that orlistat lowered plasma triglyceride levels, without effect on either total cholesterol or probably also high density lipoprotein cholesterol levels, and reduced atherosclerotic lesions in apoE KO mice fed the western-type diet. Therefore, it was experimentally suggested that not only cholesterol but also triglyceride are causative factors in the development of atherosclerosis.

Recent studies have focused on the relationship between postprandial hyperlipidemia and the development of atherosclerosis (Patsch et al., 1992; Weintraub et al., 1996). Our findings indicating that orlistat increased fecal fat excretion and reduced triglyceride level after oral fat-loading suggest that orlistat lowered postprandial triglyceride level via the inhibition of dietary fat absorption. Chylomicrons are a triglyceride-rich lipoprotein, secreted from the intestine during postprandial period and hydrolyzed to chylomicron remnants in circulation. Chylomicron remnants are known to be an atherogenic lipoprotein, not only damaging vascular endothelial function but also stimulating lipid accumulation in subendothelial macrophages (Yu and Cooper, 2001). Therefore, the triglyceride-lowering effect of orlistat shown in this study would be attributed to the reduction of chylomicron. Orlistat should lower the postprandial remnants level and consequently prevent atherosclerotic diseases. Further study to elucidate these mechanisms might be needed.

In conclusion, the present study showed that orlistat inhibited triglyceride absorption, lowered plasma triglyceride levels and reduced progression of atherosclerotic lesions in apoE KO mice fed a western-type diet. This suggests that orlistat reduces the development of atherosclerosis through a triglyceride-lowering effect via inhibition of fat absorption. Inhibition of triglyceride absorption may be a promising strategy for the prevention of atherosclerotic diseases in patients with combined hyperlipidemia, such as Type IIb and III hyperlipidemia.

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