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EFFECTS OF EBELACTONE B, A LIPASE INHIBITOR, ON INTESTINAL FAT ABSORPTION IN THE RAT

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Ebelactones A and B, natural products from *Streptomyces aburaviensis* are potent inhibitors of pancreatic lipase. Lipase is the key enzyme required for the absorption of dietary triglycerides (TG). Ebelactone B inhibited, in a dose-dependent manner, the intestinal absorption of fat after fat-feeding in the rat. The most effective inhibition was observed when the inhibitor was administered at 60 min prior to fat-feeding. When ebelactone B (10 mg/kg) was administered, the serum levels of TG (58%) and cholesterol (36%) were decreased. Since ebelactone B effectively inhibits absorption of dietary fat, it may provide a promising means for prophylaxis or therapeutics of hyperlipidemia and obesity.

KEY WORDS: Ebelactones A and B, inhibitor, lipase, esterase, triglyceride, cholesterol, hyperlipidemia, obesity

INTRODUCTION

Hyperlipidemia and obesity are caused by an abnormal accumulation of fat and are closely related to the occurrence of coronary arterial diseases, leading to a high rate of mortality.[1,2] One of the main causes of these diseases is an excessive uptake of dietary fat. Usually, the energy supplied through fat in meals constitutes 40% of the total supplied through meals, most of the fat consisting of TG.

Pancreatic lipase is the key enzyme required for the absorption of dietary TG.[3] This enzyme splits fatty acids from TG at the 1- and 3-carbon positions. The resultant free fatty acids and monoglycerides are then incorporated into bile acid-phospholipid micelles. These micelles are absorbed at the level of the brush border of the small intestine and eventually enter the peripheral circulation as chylomicrons.[4–6]

Thus it is considered that a compound that selectively limits the absorption of ingested fat could be useful in the treatment of either hyperlipidemia or obesity. It has recently been shown that tetrahydrolipstatin (THL), derived by hydrogenation

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from lipstatin, a lipase inhibitor produced by *Streptomyces toxytricini*, has been shown to inhibit fat absorption and to lower plasma cholesterol.[7–9]

Ebelactones A and B,[10] natural products from *Streptomyces aburaviensis* are potent inhibitors of pancreatic lipase and could be used for this purpose. The aim of the present investigation was to study the effect of ebelactone B on the intestinal absorption of total cholesterol (TC) and of TG.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats weighing 250–300 g were obtained from Sankyo Labo Service Corporation, Tokyo, Japan. The animals were given F1-fish meal (Funahashi Co. Ltd., Tokyo, Japan). The design of the study was as follows: after overnight fast, control animals were given olive oil (5 ml/kg) containing 10% cholesterol by oral administration. The dosages used in the active treatment were 2, 10, or 50 mg/kg of ebelactone B wih fat. At 3, 6, 9 h after the fat feed, blood from 5 rats of each group was withdrawn by cardiac puncture and serum-lipid was determined. A second experiment was carried out to examine the effect of the timing of ebelactone B administration. A single dose of ebelactone B (10 mg/kg) was administered at 30 min and 60 min prior to, or simultaneously with, administration of fat. A blood sample was taken at 6 h after fat feeding.

Inhibitor

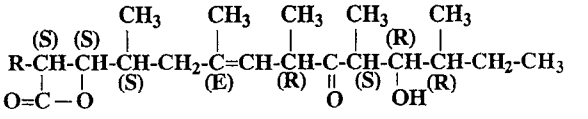
The inhibitor used in this study was ebelactone B, whose structure and activity with that of ebelactone B is shown in Table 1.

In vitro inhibitory activities of ebelactones A and B on esterase and lipase

The sources of substrates, enzymes and others were as follows: *p*-Nitrophenyl acetate (PNPA), *p*-nitrophenyl laurate (PNPL), esterase (hog liver) and lipae (hog pancreas) were obtained from Sigma Chemical Company, St., Louis, USA. Cholesterol, olive oil and Triton X-100 were obtained from Wako Pure Chem. Ind., Osaka, Japan.

For the assay of esterase, 25 μl of 4 mM *p*-nitrophenyl acetate, 100 μl of 0.1 M phosphate buffer containing 0.06% Triton X-100 (pH 5.0), 50 μl of esterase (0.1 mg/ml) and 25 μl of H_2O or inhibitor solution were dispensed into a microwell plate (nunclone, F96, Copenhagen, Denmark). For the assay of lipase, 25 μl of 4 mM *p*-nitrophenyl laurate, 100 μl of 0.1 M phosphate buffer containing 0.06% Triton X-100, 50 μl, of lipase (0.1 mg/ml) and 25 μl of H_2O or inhibitor solution were dispensed into a microwell plate. The mixtures were incubated for 1 h at 37°C, and the absorbance at 405 nm was determined using a BIO-RAD microplate reader model 3550 (Seiko-Epson Co. Ltd., Suwa, Japan). The IC_{50} values for the inhibitory activities of ebelactones A and B against esterase and lipase are shown in Table 1.

TABLE 1
Inhibitory activities of ebelactones A and B.



Ebelactone (A: R=CH_3, B:R=C_2H_5)

	IC_{50}(μg/ml)	
	Esterase	Lipase
Ebelactone A	0.056	0.003
Ebelactone B	0.0035	0.0008

Esterase: hog liver, Lipase: hog pancreas.

Assay of serum lipoprotein-cholesterol

The assays of TG, TC and PL (phospholipids) were done with a commonly used assay kits as follows: TG kit from Daiich Chem. Co., Tokyo Japan, TC kit and PL kit from Wako Pure Chem. Ind., Osaka, Japan.

RESULTS AND DISCUSSION

Effect of pH on inhibitory activity of ebelactone B in vitro

Prior to oral administration of ebelactone B, the stability of the compound in the pH range of stomach and intestine was examined (Figure 1). The inhibitory activity of ebelactone B remained rather unaffected from pH 2 to 9 but decreased sharply at pH 10.

Effect of ebelactone B administration on serum-lipase activity in vivo

Ebelactone B would be expected to remain within the intestinal tract and should not be absorbed through it. The change in lipase activity in the serum after administration of ebelactone B was measured. The animals were divided into 5 groups depending on whether they were fed with fat or not and on the doses of ebelactone B administered. Groups 1 to 4 were fed with fat, whereas group 5 was kept fasting (Figure 2). Ebelactone B was administered to groups 2 to 4 with the dose of 2, 10, or 50 mg/kg with fat. No ebelactone B was given to the fasting group (group 5). Among the ebelactone B-treated rats, the group receiving 2 mg/kg did not differ significantly when compared to the control group (group 1). When rats were given 10 or 50 mg/kg of ebelactone B, the lipase activity was almost at the same level as that observed in group 5.

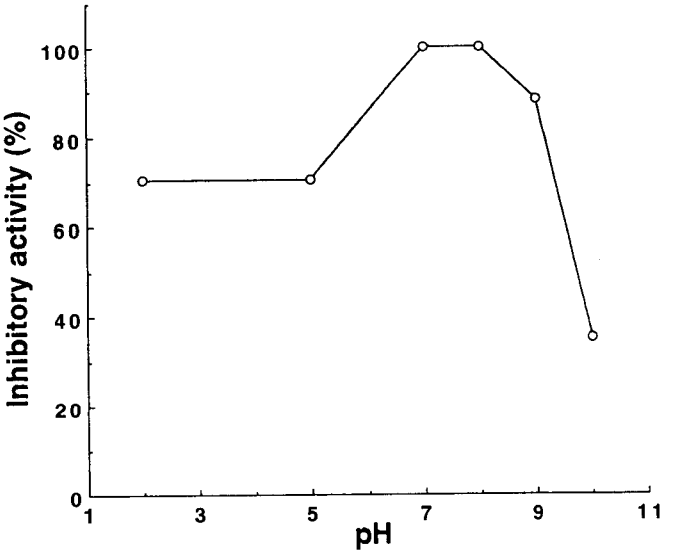


FIGURE 1 Effect of pH on *in vitro* inhibitory activity of ebelactone B towards hog pancreatic lipase. Ebelactone B was incubated for 1 h at 37°C at various pHs. IC_{50} values for ebelactone B at each pH were compared with that of the control value (pH 7.0).

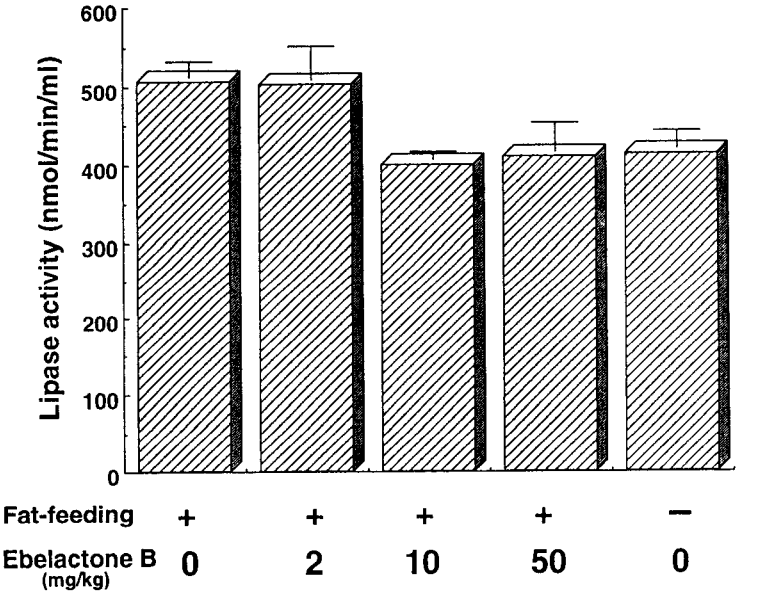


FIGURE 2 Effect of ebelactone B administration on serum-lipase activity *in vivo* in the rat.

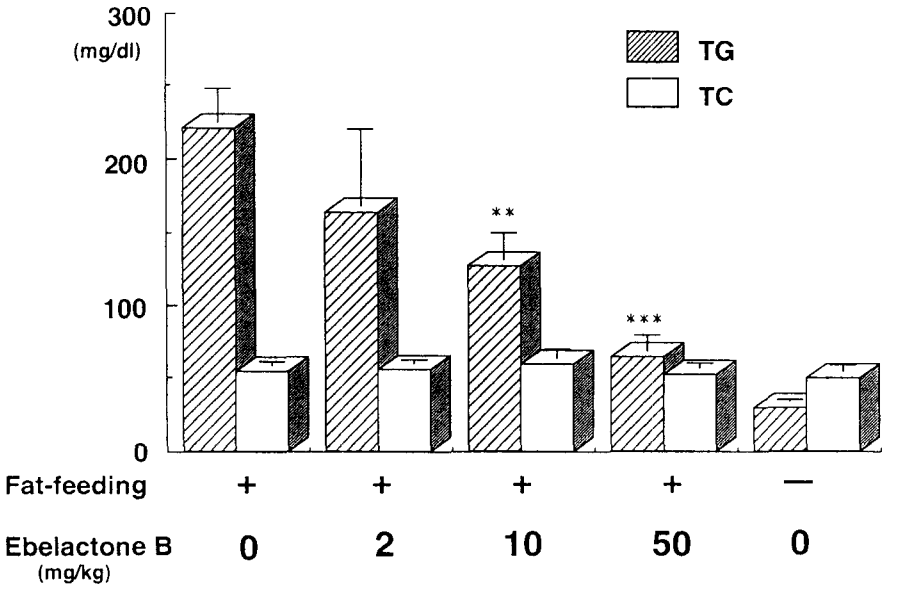


FIGURE 3 Change of TG and TC in serum from fat-feeding rat with administration of ebelactone B.

The serum lipase activity tended to be higher in groups 1 and 2 which were fat fed, but the degree of increase was not significant statistically. These results demonstrate that ebelactone B is not absorbed through the intestinal tract.

Change of TG and TC in serum from fat-feeding rat with administration of ebelactone B

After fat-feeding, the level of TL reached its maximum at 6 h and then tended to decrease toward 9 h. The effect of administration of various doses of ebelactone B on the serum fat level at 6 h after fat-feeding was examined. As shown in Figure 3, TG level in serum showed a significant dose-dependent decrease to ebelactone B. On the contrary, there was no significant change in serum TC or in serum PL.

Change in serum lipid depending on the timing of ebelactone B administration

As described previously, ebelactone B significantly suppressed the level of serum lipid when administered simultaneously with fat-feeding. In this experiment, ebelactone B was administered at 30 min or 60 min prior to fat-feeding and the resultant changes in the level of serum lipid were examined. Table 2 shows that serum TL decreased highly significantly when ebelactone B (10 mg/kg) was given at 60 min prior to fat-feeding. In addition, serum TG, TC and PL also showed a significant decrease when ebelactone B was given at 60 min prior to fat-feeding.

TABLE 2
Change of serum lipid from fat-feeding rat after ebelactone B administration at different times.

Treatment	Dose (mg/kg)	Administration time(min)	Mean±S.E. (mg/dl) (n=4) TL	TG	TC	PL
Control	0	0	391.6±22.5	220.0±23.5	53.8±2.5	91.0±4.7
Ebelactone B	10	0	310.8±24.4**	126.8±18.1**	59.0±5.9	95.5±8.7
	10	−30	273.8±23.0**	127.5±26.2**	46.3±3.8*	80.5±5.9
	10	−60	200.1±21.0***	91.7±14.5***	34.5±2.0***	59.5±4.2**

*p<0.05, **p<0.01, ***p<0.001: according to the students *t*-test. Serum level at 6 h after fat-feeding.
Abbreviations: TL, total lipid; TG, triglyceride; TC, total cholesterol; PL, phospholipid.

TABLE 3
Change of serum lipoprotein cholesterol from fat-feeding rat after ebelactone B administration at different times.

Treatment	Dose (mg/kg)	Administration time(min)	Mean±S.E. (mg/dl) (n=4) HDL-C	LDL-C	VLDL-C
Control	0	0	43.2±3.5	4.6±1.6	21.7±3.7
Ebelactone B	10	0	42.2±5.3	4.6±1.8	23.1±7.5
	10	−30	45.1±5.2	2.8±0.8	14.9±0.4*
	10	−60	35.2±4.1*	2.1±0.2*	9.5±1.7***

*p<0.05, ***p<0.001; according to the student's *t*-test. Serum level at 6 h after fat-feeding.

Change in serum lipoprotein-cholesterol depending on the timing of ebelactone B administration

Table 3 shows the changes in cholesterol fractions from several lipoproteins. Both HDL-C and LDL-C decreased significantly if ebelactone B (10 mg/kg) was given at 60 min prior to fat-feeding.

We conclude from the above results that ebelactone B (10 mg/kg) significantly suppressed the absorption of fat after fat-feeding by inhibiting pancreatic lipase when the compound was administered at 60 min prior to fat-feeding. The compound seems to provide a promising means of prophylaxis and therapeutics or hyperlipidemia and/or obesity.

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