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# ANTILIPOLYTIC EFFECT OF *p*-MYRISTYLOXY-α-METHYLCINNAMIC ACID (LK-903 ACID) AS A MECHANISM OF THE HYPOLIPIDEMIC ACTION OF ITS α-MONOGLYCERIDE (LK-903)

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**Abstract**—In a study of the mechanism of hypolipidemic action of α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903), the hydrolyzed cinnamic acid moiety (LK-903 acid) was found to inhibit *in vitro* the lipolysis manifested by rat epididymal adipocytes. Its antilipolytic action appears to be the result of inhibition of cyclic AMP formation in the fat cells in analogy with the mechanism of feedback inhibition of lipolysis by free fatty acids.

Hypolipidemic properties of α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903) were described in the preceding paper [1]. The observation that LK-903 lowered plasma free fatty acids (FFA) in the rat suggested that its mechanism of action is inhibition of lipolysis in the adipose tissue, thus limiting the supply of fatty acids for hepatic triglyceride synthesis and hence the hepatic secretion of plasma lipoproteins (VLDL).

The present paper describes the results of studies on the effect of LK-903 and its free acid (LK-903 acid) on rat epididymal fat cells *in vitro*. The results indicate that LK-903 acid inhibits catecholamine- and theophylline-induced lipolysis by suppressing the accumulation of cyclic AMP in analogy with the inhibition of lipolysis by long-chain fatty acids [2, 3], clofibric acid [4] and nicotinic acid [5].

## MATERIALS AND METHODS

*Animals.* Male Sprague–Dawley rats were purchased from Nihon CLEA Co., Tokyo, and maintained on laboratory chow (Nihon CLEA CE-2 pellets) at least for one week before use. The methods of grouping the rats, administering the test compound and sampling the blood were described previously [6].

*Chemicals.* α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903) and α-methyl-*p*-myristyloxy cinnamic acid (LK-903 acid) were synthesized by Watanabe *et al.* [7] at the Products Formulation Research Laboratory of Tanabe Seiyaku Co. Clofibric acid was obtained by hydrolysis of clofibrate in ethanolic KOH and recrystalization in water (m.p. 118 ~ 120°). Theophylline and dibutyryl cAMP were purchased from Nakarai Chemicals Ltd., epinephrine from E. Merk Japan Ltd., and norepinephrine from Tokyo Kasei Kogyo Co.

*Analytical methods.* The methods for determination of total cholesterol [8], triglyceride [9] and FFA [10] are the same as in the preceding paper [1]. Glycerol was determined by the method of Laurell and Tibbling [11]. Cyclic AMP was determined by Gilman's binding assay [12] after the procedure described by Wombacher and Körber [13] with the rat liver binding protein purified by the method of Kumon *et al.* [14].

In vitro *experiments.* Rats weighing 300 ~ 400 g were killed by decapitation and the epididymal fat pads were excised. Adipocytes were prepared by the method of Rodbell [15]. The adipocytes were suspended in 2 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 55 mM $CaCl_2$, 2% bovine serum albumin (Sigma, Fraction V), lipolytic and antilipolytic agents in a plastic tube, and incubated at 37° under the gas phase of 95% $O_2$–5% $CO_2$. The test compounds (LK-903, LK-903 acid, clofibric acid) were suspended in the above medium with Nikkol HCO 60 (hydrogenated caster oil, final concentration 0.05%). After periods of time (usually one hour) incubation mixtures containing the fat cells were cooled in ice water and used for the assays of glycerol and cyclic AMP.

## RESULTS

Administration of 0.02% LK-903 in the diet for one week has been effective in reducing serum cholesterol and triglyceride in rats, and 0.03% LK-903 for one month has caused lowerings of serum phospholipid and FFA as well [1]. The present study, in which 0.2% LK-903 was administered in the diet for 3 days, showed that the circulating level of serum glycerol was also lowered, suggesting that LK-903 inhibited lipolysis in the adipose tissue *in vivo* (Fig. 1).

Since LK-903 (monoglyceride) is expected to undergo hydrolysis in the body to yield α-methyl-*p*-myristyloxycinnamic acid (LK-903 acid) and glycerol, both LK-903 and LK-903 acid were tested for their possible inhibitory effects on the epinephrine-induced lipolysis by rat epididymal adipocytes. When lipolysis was induced by 0.5 μg/ml ($2.7 \times 10^{-6}$ M) of epine-

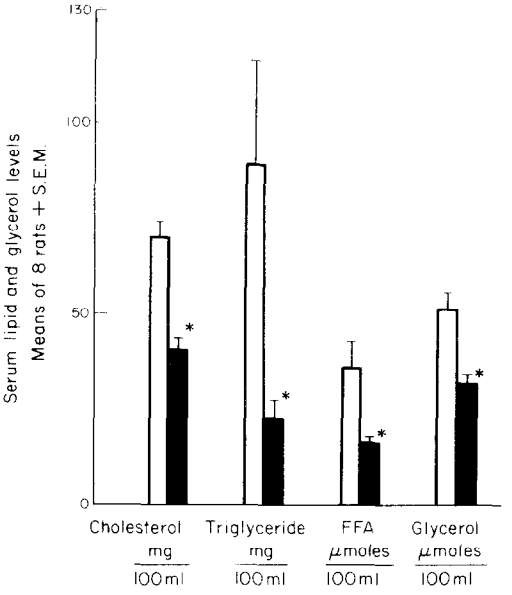


Fig. 1. Effect of administration of LK-903 on serum lipids and glycerol in rats.

Blood samples were taken from the rats after the feeding period of 3 days. The experimental group (filled columns) was given a diet containing 0.2% LK-903. An asterisk denotes a significant difference ($P < 0.05$ or better) from the control group (open columns).

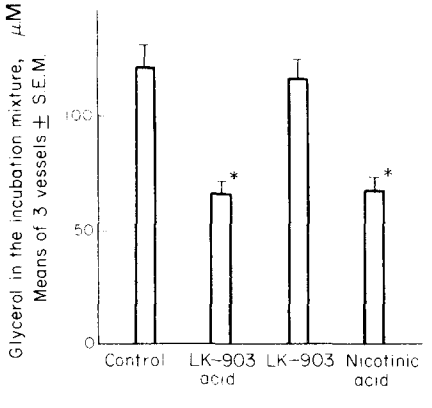


Fig. 2. Effects of LK-903, LK-903 acid and nicotinic acid on epinephrine-induced lipolysis by rat epididymal adipocytes.

Rat epididymal adipocytes were incubated in the presence of epinephrine (0.5 μg/ml) and a test compound (0.5 mg/ml) for 1 hour. An asterisk denotes a significant difference ($P < 0.01$) from control.

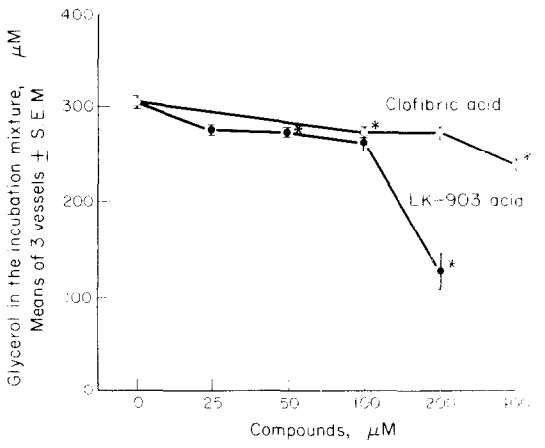


Fig. 3. Antilipolytic effects of LK-903 acid and clofibric acid in rat epididymal adipocytes.

Rat epididymal adipocytes were incubated in the presence of epinephrine ($10^{-6}$ M) and various concentrations of LK-903 acid or clofibric acid for 1 hr. An asterisk denotes a significant difference ($P < 0.01$) from control.

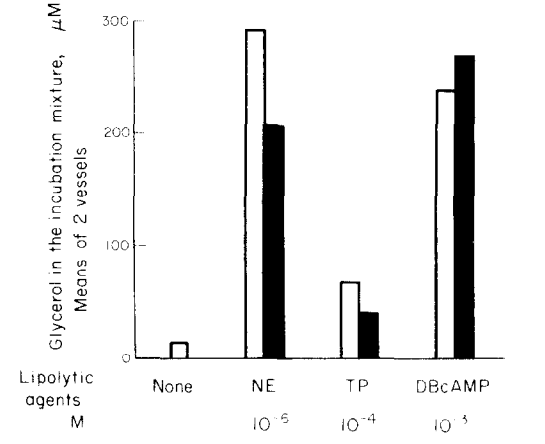


Fig. 4. Effect of LK-903 on norepinephrine (NE)-, theophylline (TP), and dibutyryl cyclic AMP (DBcAMP)-induced lipolysis by rat epididymal adipocytes.

Rat epididymal adipocytes were incubated in the presence (filled columns) or absence (open columns) of $10^{-3}$ M LK-903 acid for 1 hr under the various lipolytic conditions denoted on the graph.

phrine and measured by glycerol release, addition of 0.5 mg/ml ($1.3 \times 10^{-3}$ M) of LK-903 acid inhibited the lipolysis nearly 50 per cent while LK-903 itself was not inhibitory (Fig. 2). Nicotinic acid at the concentration of 0.5 mg/ml brought about an inhibition of the same magnitude as by 0.5 mg/ml LK-903 acid. It should be kept in mind that even partial hydrolysis of LK-903 would produce free glycerol in amounts high enough to obscure the result. This possibility, however, is unlikely because the glycerol release in the presence of LK-903 never exceeded that of the control vessel under a variety of conditions (with various LK-903 and cell concentrations).

Concentration–inhibition relationships were studied with LK-903 acid and clofibric acid (Fig. 3) in the presence of a lower concentration ($10^{-6}$ M) of epinephrine. A slight but significant inhibition was observed even at as low as $2.5 \times 10^{-5}$ M of LK-903 acid. Inhibition became quite pronounced at $2.0 \times 10^{-4}$ M. At this concentration, clofibric acid was only slightly inhibitory.

Effect of LK-903 acid on variously induced lipolysis was examined (Fig. 4). LK-903 acid inhibited norepinephrine- and theophylline-induced lipolysis, but it showed no inhibitory effects on dibutyryl cyclic AMP-induced lipolysis, suggesting that LK-903 acid might inhibit lipolysis by interfering with the production of cyclic AMP in the cells. In order to test

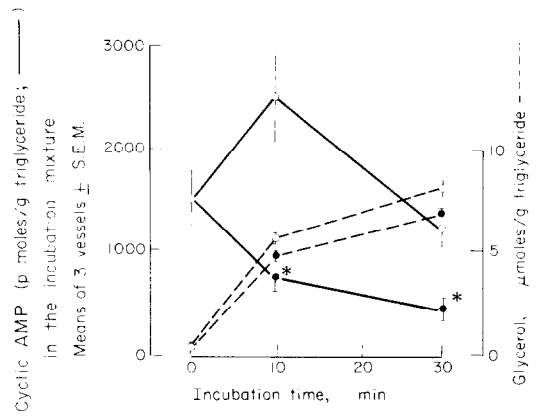


Fig. 5. Effects of LK-903 acid on norepinephrine-induced lipolysis and cyclic AMP accumulation in rat epididymal adipocytes.

Rat epididymal adipocytes were incubated in the presence of $10^{-6}$ M norepinephrine and $10^{-4}$ M theophylline with (filled circles) or without (open circles) $10^{-3}$ M LK-903 acid for 10 and 30 min. An asterisk denotes a significant difference ($P < 0.05$ or better) from control.

this possibility, rat fat cells were incubated in the presence of norepinephrine and theophylline with or without LK-903 acid, and the cyclic AMP concentration of the whole incubation mixture was determined at 10 and 30 min. The cyclic AMP contents of the vessels containing LK-903 acid were much lower than those of the control vessels (Fig. 5).

## DISCUSSION

LK-903 acid behaves like a long-chain fatty acid in the determination by the method of Itaya and Ui and gives false positive coloration. Therefore, if LK-903 is hydrolyzed in the body into LK-903 acid and glycerol, determination of serum FFA and glycerol could theoretically give higher values. However, LK-903, when administered to rats, invariably depressed serum FFA even at the highest dose [1]. The present study showed that depression of serum glycerol accompanied the lowering of FFA indicating that LK-903 depressed plasma FFA by inhibiting fat mobilization and not by stimulating FFA utilization. This inhibition of fat mobilization would restrict the supply of FFA for triglyceride synthesis in the liver and in turn lower the hepatic content of triglyceride and the secretion of VLDL into plasma [1].

The inhibition of lipolysis by LK-903 acid was verified *in vitro* in the present experiments. This inhibition probably arises as a result of inhibition of the production of cyclic AMP by adenylate cyclase in the fat cells (Fig. 5). The same mechanism of action has been proposed for the two hypolipidemic drugs clofibrate [4] and nicotinic acid [5].

The inhibition of lipolysis through inhibition of adenylate cyclase has also been suggested as the mechanism of feedback inhibition of lipolysis by free fatty acids [2, 3]. In this respect LK-903 acid would be considered as a false feedback inhibitor of lipolysis. In fact LK-903 acid seems to be mistaken as a fatty acid by the cell and found incorporated into triglycerides of plasma [16] and chyle [17] after its ingestion. The reason why inhibition of *in vivo* lipolysis takes place in the fat cells laden with LK-903 acid-containing fat might be that LK-903 seems to be more potent than natural fatty acids as feedback inhibitor, since it inhibited lipolysis markedly at the concentration of 0.2 mM (Fig. 3) while a comparable inhibition of lipolysis or cyclic AMP production would require 10 times higher concentrations of sodium oleate [2, 3]. If the above mechanism proves to be correct, LK-903 (and its acid) could be useful as an agent that inhibits lipolysis in a physiological manner.

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