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HYPOLIPIDEMIC EFFECT OF α-MONO-*p*-MYRISTYLOXY-α′-METHYLCINNAMOYL GLYCEROL (LK-903) IN RATS

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Abstract—The hypolipidemic effect of an α-monoacylglycerol analog, α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903), was studied in rats. Its hypolipidemic activity in normal rats was about twice that of clofibrate. It did not produce hepatomegaly. Its free acid form, *p*-myristyloxy-α-methylcinnamic acid, was also active, although less active than the monoglyceride form. LK-903 was effective in fructose-, cholesterol-, and Triton-induced hyperlipidemias and phenobarbital-induced fatty liver. LK-903 differs from clofibrate in that it invariably depresses the triglyceride concentration of the liver. Its pharmacological effect seems to be ascribable to the depression of circulating free fatty acids.

Clofibrate is currently the most widely used drug for the treatment of hyperlipidemias, but its therapeutic as well as prophylactic effects on atherosclerotic diseases are still controversial [1]. Its serious drawback is its relative inefficacy in Type IIa hyperlipidemia.

The present investigation was initiated to search for more satisfactory hypolipidemic agents. In the course of screening studies with rats a series of *p*-alkyloxy-substituted cinnamic acid derivatives were found to possess potent hypocholesterolemic activities. Out of these compounds was selected α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903) for further studies on the basis of its therapeutic index. This report deals with detailed studies of its hypolipidemic effect in rats in comparison with clofibrate. The features of this hypolipidemic agent are its structural similarity to α-monoglyceride and its depressing effects on circulating free fatty acids (FFA) and liver triglyceride.

During the preparation of the manuscript of this paper, a report on hypolipidemic activities of *p*-myristyloxy-cinnamic acid methyl ester and other fatty acid-like alkyloxyarylcarboxilic acids has appeared [2].

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats were purchased from Nihon CLEA Co., Tokyo, and maintained on commercial laboratory chow (Nihon CLEA CE-2 pellets) for at least 1 week before use.

Grouping of the rats, blood sampling, and calculation of the hypolipidemic effect were performed as described previously [3]. When administered in the diet, the drugs were thoroughly ground in a mortar manually with a small amount of powder chow (Nihon CLEA CE-2 powder), diluted to a desired concentration by mixing with a mechanically driven pestle in a larger mortar, and fed *ad lib.* In the long term experiments, rats were bled from the abdominal aorta under ether anesthesia and the liver was excised.

Determination of serum and liver lipids. The methods for determination of total cholesterol [4], triglyceride [5], and phospholipid [6] in serum and liver are given in the previous paper. Serum FFA was determined by the method of Itaya and Ui [7].

Chemicals. α-mono-*p*-myristyloxy-α′-methylcinnamoyl glycerol (LK-903) and its free acid (LK-903 acid) were synthesized by Watanabe *et al.* [8] at the Products Formulation Research Laboratory of Tanabe Seiyaku Co., Ltd. Triton WR-1339 was obtained from Rohm and Haas Co. The hydrogenated fat used for the fatty liver experiment was Cocolin (baker's shortening) of Taiyo Yushi Co., Ltd., Yokohama.

RESULTS

Effects on serum and liver lipid concentrations in normal male rats. The hypolipidemic activities of LK-903, LK-903 acid and clofibrate are expressed in mean percent decreases in the concentration of serum cholesterol and triglyceride of rats which were maintained for one week on the experimental diets as shown in Table 1. LK-903 is effective at a dietary concentration of 20 mg per 100 g of the powder chow, equivalent to about 20 mg per kg body weight per day. This activity is roughly twice that of clofibrate. Its free acid (LK-903 acid) is less active than the α-monoacylglycerol form. Serum triglyceride was lowered by all the three drugs to a greater extent than serum cholesterol.

Effects of the addition of LK-903 and clofibrate in the diet for one month on serum lipids (cholesterol, triglyceride, FFA and phospholipid) and liver lipids (cholesterol, triglyceride and phospholipid) are shown in Tables 2 and 3. The two drugs depressed all the serum lipids. Among the liver lipids, triglyceride was lowered markedly by LK-903, but not by clofibrate. The latter drug depressed the concentration of liver cholesterol, while it elevated liver phospholipid. Unlike clofibrate, LK-903 did not produce hepatomegaly in the rat (Table 3).

It was noted that LK-903 was more effective when

Table 1. Mean percent decreases in serum lipid concentrations in normal male rats treated with LK-903, LK-903 acid and clofibrate

Drugs % in diet for 7 days	Serum lipids mg/100 ml Control	Mean % decrease in serum lipids* LK-903 0.02	LK-903 0.05	LK-903 0.1	LK-903 acid 0.02	LK-903 acid 0.05	LK-903 acid 0.1	Clofibrate 0.05	Clofibrate 0.1
Cholesterol	84 ± 1 (25)†	14 ± 3 (8)	25 ± 1 (14)	45 (1)	12 ± 4 (4)	17 ± 2 (7)	16 (2)	14 ± 5 (3)	22 ± 3 (8)
Triglyceride	79 ± 3 (25)	33 ± 6 (8)	43 ± 6 (14)	52 (1)	38 ± 8 (4)	26 ± 8 (7)	45 (2)	35 ± 16 (3)	41 ± 6 (8)

* Means ± S.E.M.
† The number of experiments performed is given in parentheses.

Table 2. Effects of LK-903 and clofibrate on serum lipid concentrations in rats

	Dose of compounds (% in diet for one month)	Serum lipids* Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Phospholipid (mg/100 ml)	FFA (μmoles/100 ml)
None	—	79 ± 5	79 ± 8	153 ± 7	189 ± 8
LK-903	0.03	63 ± 3§	53 ± 6§	123 ± 5‖	167 ± 4‡
	0.1	45 ± 3¶	26 ± 3¶	80 ± 5¶	170 ± 7†
	0.3	41 ± 3¶	31 ± 3¶	70 ± 3¶	166 ± 9†
Clofibrate	0.1	48 ± 3¶	47 ± 4‖	101 ± 5¶	134 ± 8¶
	0.3	39 ± 3¶	35 ± 2‖	86 ± 3¶	129 ± 11¶

* Means of 10 rats per group ± S.E.M. (initial body weight approx. 130 g).
† $P < 0.1$.
‡ $P < 0.05$.
§ $P < 0.02$.
‖ $P < 0.01$.
¶ $P < 0.001$.

Table 3. Effects of LK-903 and clofibrate on liver lipid concentrations and liver weight in rats

	Dose of compounds (% in diet for one month)	Liver lipids (mg/g)* Cholesterol	Triglyceride	Phospholipid	Liver weight (g/rat)*
None	—	3.18 ± 0.09	8.05 ± 0.59	33.2 ± 0.8	13.85 ± 0.34
LK-903	0.03	3.19 ± 0.11	6.63 ± 0.98	32.0 ± 0.5	13.80 ± 0.56
	0.1	2.98 ± 0.09	4.45 ± 0.41§	31.8 ± 0.8	14.05 ± 0.46
	0.3	2.78 ± 0.10‡	5.33 ± 0.29§	34.3 ± 1.5	14.03 ± 0.33
Clofibrate	0.1	2.97 ± 0.06†	7.93 ± 1.12	37.5 ± 0.6§	13.58 ± 0.31
	0.3	2.61 ± 0.06‡	7.18 ± 0.76	40.5 ± 0.5§	16.38 ± 0.67‡

* Means of 10 rats per group ± S.E.M. (initial body weight approx. 130 g).
† $P < 0.1$.
‡ $P < 0.01$.
§ $P < 0.001$.

it was administered in the diet than when given once a day with stomach tube. If a drug is added to the diet and ingested by the rats *ad lib.*, more steady blood concentrations of the drug would be maintained than when the drug is administered once a day. In order to give less fluctuation in the blood concentration of LK-903, groups of fasted rats were administered a suspension of LK-903 with stomach tube in three divided doses at four-hour intervals. For comparison, other groups of fasted rats were placed on diets containing LK-903 in such concentrations that the rats should consume in each 4 hr the same doses of LK-903 as the former groups of rats received through stomach tube. The doses of 100 and 300 mg/kg of LK-903 were high enough to exert the lipid-lowering effect within 24 hr in either mode of administration. The hypolipidemic effect was again greater when the drug was incorporated into the diet (Fig. 1).

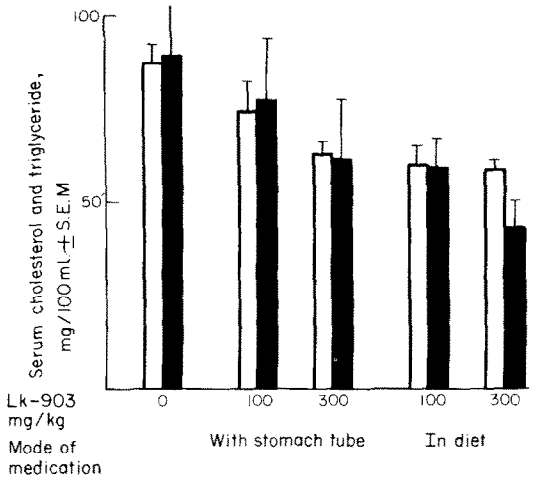

Fig. 1. Difference in the efficacy of LK-903 between the two modes of administration.

After overnight fasting, rats (ca. 400 g, 10 rats per group) were administered 100 and 300 mg/kg LK-903 in three divided doses at 4 hr intervals either by stomach tube or in the diet. The quantity of powder chow for the diet was restricted to insure complete consumption within each 4 hr interval. All the groups of rats received the same amounts of food (either basal diet or LK-903 diet) and Nikkol solution by stomach tube under the same time schedule until the third interval and then fed *ad lib.* during the 4th interval, after which all the rats were bled for the determination of serum cholesterol (open column) and triglyceride (closed column).

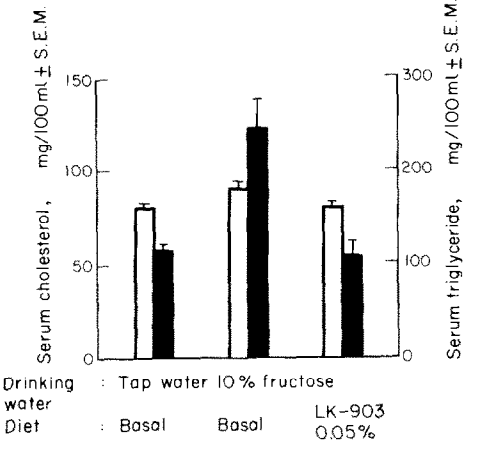

Fig. 2. Hypolipidemic effect of LK-903 on fructose-induced hyperlipidemia in rats.

Hyperlipidemia was induced by giving 10% fructose in drinking water *ad lib.* for 2 weeks to two groups of 10 rats (ca. 115 g); one group was placed on a diet containing 0.05% LK-903 and the other on the basal diet for this same period. Serum cholesterol (open column) and triglyceride (closed column) were determined at the end of the 2-week period.

Effect on experimentally induced hyperlipidemias and fatty liver in rats. Inclusion of 10% fructose in drinking water is known to elevate serum triglyceride in rats [9]. In our experiment the mean concentration of serum triglyceride of the rats supplied with 10% fructose for 2 weeks was about twice that of control rats. This increase in serum triglyceride was completely prevented by simultaneous administration of 0.05% LK-903 in the diet (Fig. 2). A slight, concurrent increase in serum cholesterol by the treatment with 10% fructose was also prevented by LK-903.

The dietary hypercholesterolemia in the rat was also ameliorated by inclusion of LK-903 in the diet. Addition of 2% cholesterol and 1% sodium cholate in the diet caused a three- to four-fold increase in serum cholesterol in one week. On continuation of the same diet for another week there was some, but statistically insignificant, decrease in serum cholesterol, while supplementation of this hypercholesterolemic diet with either 0.1% LK-903 or 0.2% clofibrate during the second week brought the pre-medication level of serum cholesterol down to nearly a half (Table 4). On the other hand, the hepatic cholesterol level was not significantly affected by the medication with LK-903, while clofibrate significantly elevated the total cholesterol content of the liver (Table 4).

LK-903 and clofibrate were examined in Triton-induced hyperlipidemia in rats. Triton WR-1339 is known to interfere with the removal of triglyceride from the circulation [10] and therefore the rate of plasma lipid accumulation after the intravenous injection of Triton reflects the rate at which the lipids are entering the circulation. As shown in Fig. 3 these two drugs, when fed to rats for 3 days prior to the injection of Triton, greatly inhibited the accumulation of both

Table 4. Effects of LK-903 and clofibrate on dietary hypercholesterolemia in rats

Additions to the hypercholesterolemic diet†		Serum cholesterol* mg/100 ml		Liver cholesterol* mg/g liver	Liver cholesterol* mg/liver
		Day 7	Day 14	Day 14	Day 14
None	–	334 ± 39	274 ± 22	5.1 ± 0.3	62.1 ± 3.9
LK-903	0.1%	353 ± 31	181 ± 18§	5.5 ± 0.2	66.5 ± 4.4
Clofibrate	0.2%	334 ± 24	149 ± 8‖	4.9 ± 0.2	74.1 ± 3.1‡

* Means of 10 rats per group ± S.E.M.

† Rats (ca. 130 g) were placed on the hypercholesterolemic diet containing 2% cholesterol and 1% sodium cholate for 2 weeks. The drugs were administered in the diet during the second week.

‡ $P < 0.05$.

§ $P < 0.01$.

‖ $P < 0.001$.

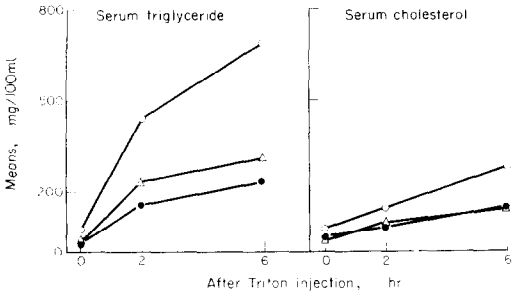

Fig. 3. Hypolipidemic effects of LK-903 and clofibrate on Triton-induced hyperlipidemia in rats.

Rats (ca. 200 g, 8 rats per group) were fed the control diet (open circle) or experimental diet (closed circle, 0.2% LK-903; open triangle, 0.2% clofibrate) for 3 days before the intravenous injection of Triton WR-1339 (225 mg/kg), and blood samples were obtained from the tail tip at the times indicated on the graph.

serum triglyceride and cholesterol, suggesting that the hepatic secretion of plasma lipids was greatly reduced under the influence of these drugs.

Since LK-903 has a marked depressive effect on liver triglyceride, it was expected that this drug might prevent the development of fatty liver. The phenobarbital-induced fatty liver described by Sorrell *et al.* [11] was chosen as the experimental model of fatty liver. Repeated i.p. injection of phenobarbital for three consecutive days to rats on a high-fat diet resulted in a "fatty liver" whose triglyceride content was 2 to 3 times higher than the control level. Inclusion of 0.2% LK-903 in the high-fat diet during the above treatment period depressed this elevation of hepatic triglyceride by 31% (Table 5).

DISCUSSION

LK-903 is unique as a hypolipidemic agent, for its structure resembles α-mono-fattyacylglycerol (α-monoglyceride). Since LK-903 acid is also active, it is likely that LK-903 exerts its effect after hydrolysis in the body. However, the possibility that LK-903 may act as such or in other forms is not completely excluded because it was found by Mizobe and Kohno that LK-903 acid behaved as a false fatty acid and was incorporated into mono-, di-, and triglycerides after absorption from the gut [12]. The reason why LK-903 is more effective than LK-903 acid is unknown. Judging from the blood concentration of LK-903 derivatives after oral administration, Mizobe and Kohno observed that LK-903 was more readily absorbed than its free acid (LK-903 acid) when orally administered to beagle dogs in the form of pure powder or tablets [12]. They also demonstrated that intestinal absorption of LK-903 was more efficient in fed dogs than in fasted dogs [12]. This latter observation seems to correlate with our observation that LK-903 was more effective when given to rats as a constituent of the diet than when administered in suspension through stomach tube (Fig. 1). Probably LK-903 is more thoroughly dispersed, and therefore more efficiently absorbed, in the gut by grinding it with powder chow in a mortar before administration than by administering it in suspension and letting it mix with the intestinal contents inside the gut. In this respect LK-903 may be more easily dispersed to form micelles than LK-903 acid, since monoglycerides of long-chain fatty acids are well-known emulsifying agents, the 2-monoacyl glycerols are the major end product of fat digestion [13, 14].

LK-903 lowers serum FFA in rats. The possibility that this depression of serum FFA is due to an inhibition by LK-903 of lipolysis at the adipose tissue has been explored in *in vitro* experiments [15], and we have obtained evidence indicating that LK-903 acid inhibits epinephrine-induced lipolysis by rat epididymal adipocytes by a mechanism analogous to that of the inhibition of lipolysis by fatty acids (inhibition of adenylate cyclase) [16]. It is most likely that a shortage in supply of circulating FFA to the liver would limit the rate of hepatic triglyceride formation, resulting in the decrease in hepatic triglyceride contents followed by the attenuated secretion of VLDL into the circulation. Clofibrate also depressed the circulating FFA (Table 2), [17] and this has been ascribed to the inhibition of lipolysis by this agent by way of inhibition of adenylate cyclase [18]. This sequence of events leading to the substrate deprivation for hepatic lipoprotein synthesis has been

Table 5. Effect of LK-903 on phenobarbital-induced fatty liver in rats

Additions to the basal diet for 3 days†	PB injection	Serum lipids mg/100 ml* Cholesterol	Serum lipids mg/100 ml* Triglyceride	Liver lipids mg/g* Cholesterol	Liver lipids mg/g* Triglyceride
None	—	72 ± 3	38 ± 10	3.13 ± 0.12	7.0 ± 0.5
LK-903 (0.2%)	—	30 ± 4¶	20 ± 3	3.28 ± 0.18	4.8 ± 0.6‖
Hydrogenated fat (29%)	+	70 ± 3	91 ± 11	3.43 ± 0.11	17.6 ± 2.2
Hydrogenated fat (29%) and LK-903 (0.2%)	+	48 ± 3¶	53 ± 13§	3.73 ± 0.15	12.1 ± 1.4‡

* Means of 8 rats ± S.E.M.

† Fatty liver was induced by injecting i.p. to rats 50 mg/kg sodium phenobarbital (PB) twice a day for 3 days while the rats were maintained on the fat-rich diet. In the experimental groups LK-903 was administered in the diet during the 3 days.

‡ $P < 0.1$, § $P < 0.05$, ‖ $P < 0.02$, ¶ $P < 0.001$ (in comparison with the respective non-medicated group.)

proposed as a mechanism of the hypolipidemic effect of this drug [18].

Differences were observed between the effects of LK-903 and clofibrate on liver lipid concentration. Although LK-903 invariably reduced liver triglyceride concentration, clofibrate was without effect (Table 3). Previous workers observed an increase [19–21], a decrease [22] and no change [23] in the triglyceride concentration of rat liver when 0.2 to 0.3% clofibrate was administered in the diet. Clofibrate has been reported to inhibit not only the formation of liver triglyceride [20, 24] but also the secretion of lipoproteins from the liver [25]. This latter effect would counteract the triglyceride-lowering effect and shift the balance toward accumulation.

Clofibrate tended to lower the liver concentration of cholesterol while it elevated that of phospholipid in treated rats. On the other hand, these lipid concentrations were not markedly affected by LK-903 (Table 3). The decrease in liver cholesterol and the increase in liver phospholipids caused by clofibrate have been reported by other workers [25, 26]. The former effect could be ascribed to an inhibition of hepatic cholesterol synthesis [27] and the latter to a proliferation of intracellular membranes accompanying hepatomegaly [23, 26, 28]. LK-903 probably has no or only a weak action to induce these phenomena in the rat liver.

Fructose-induced hypertriglydemia has been ascribed to high rates of conversion of this hexose to hepatic fatty acids [29] and triglyceride glycerol [30] leading to an accumulation of triglycerides in the liver [29]. This accumulation would be counterbalanced by a reduction of hepatic triglyceride synthesis from plasma FFA in fructose-fed, LK-903-treated rats, which probably explains their apparently normal serum triglyceride levels as shown in Fig. 2.

Triton-induced hyperlipidemia has been used as a test system to study the rate of hepatic secretion of plasma lipids and to demonstrate inhibition of this process by carbon tetrachloride [31], 4-aminopyrazolopyrimidine [32] and orotic acid [33]. Since the hepatic contents of triglyceride should have been greatly reduced by LK-903 by the time Triton was injected, the inhibition of accumulation of plasma triglyceride (and hence plasma cholesterol) after Triton injection would be a natural consequence (Fig. 3). On the other hand, clofibrate, which does not necessarily lower hepatic triglyceride contents, could have inhibited the process of VLDL secretion itself as suggested by Gould *et al.* [25]. The hypocholesterolemic effects of LK-903 and clofibrate in dietary hypercholesterolemia in the rat (Table 4) can be explained by these decreases in plasma lipoprotein secretion from the liver in the treated rats.

The consistent lowering of hepatic triglyceride contents by LK-903 points to a possibility that this compound might be useful as an agent to reduce fat load of fatty livers in general. The partial prevention of phenobarbital-induced fatty liver by LK-903 (Table 5) probably reflects a limiting supply of plasma FFA in LK-903-treated rats.

In conclusion, LK-903 and clofibrate may share a common mechanism (FFA depression due to the inhibition of fat mobilization) for their hypolipidemic effects, but differ in that LK-903 consistently lowers hepatic contents of triglyceride and does not produce hepatomegaly in the rat. In addition to these merits as a therapeutic, the structural similarity of LK-903 to α-monoglyceride and the behavior of LK-903 acid as a false fatty acid give impetus to further studies on this hypolipidemic agent.

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