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Hypocholesterolemic Activities of LK-903 and Clofibrate in Miniature Pigs, Cynomolgus Monkeys and Beagle Dogs

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The hypocholesterolemic activities of LK-903 (α -methyl-*p*-myristyloxycinnamic acid-1-monoglyceride) and clofibrate were examined in miniature pigs (OHMINI-875) and beagle dogs. When administered in the diet for one week, LK-903 and clofibrate were active in the miniature pig at doses of 5 and 10 mg/kg/day, respectively, whereas the two drugs showed no hypocholesterolemic activity in the beagle dog. In cynomolgus monkeys, LK-903 showed a hypocholesterolemic effect, but serum cholesterol depression was less marked than in the miniature pig.

Keywords—hypocholesterolemic activity; miniature pig; monkey; dog; clofibrate; serum cholesterol; pre-clinical study; species difference; cinnamic acid derivative; monoglyceride

α -Methyl-*p*-myristyloxycinnamic acid-1-monoglyceride (LK-903) is a novel hypolipidemic agent,²⁾ which has been selected for further developmental studies out of a number of analogous compounds which were found active in a screening system with rats. As a prerequisite to human studies, its efficacy and toxicity were examined in larger experimental animals. This paper reports the results of tests of the hypocholesterolemic activities of LK-903 and clofibrate in miniature pigs, cynomolgus monkeys and beagle dogs.

Experimental

Male miniature pigs (OHMINI-875)³⁾ were purchased from Nippon Research Laboratory for Domestic Animals (Ishibashi-cho, Tochigi) and maintained on standard chow rations (Nihon CLEA CS-17 pellets, 1/20 of body weight per day until 3.5 months of age, 1/30 until 6 months, and 1/40 thereafter). At weekly intervals, blood samples were taken from an earlobe vein and their serum cholesterol levels were determined by the method of Zak *et al.*⁴⁾ Pigs whose serum cholesterol levels appeared stable for several weeks were chosen as experimental animals and the remainder served as control animals. The experimental animals were fed a drug-containing diet twice daily (at 9 a.m. and 4 p.m.), generally for one week. The drugs were evenly mixed with a portion of powdered chow and added to the rations immediately before feeding. After the medication period the pigs were fed the standard rations until ready for another testing period. In this way all the pigs went through alternating periods of testing and control. Drug effects were expressed as percent decreases in serum cholesterol, which were calculated from the cholesterol concentrations determined immediately before and after a medication period.

Cynomolgus monkeys were purchased from Kasho Co., Ltd. (Tokyo) and maintained on the basal diet for monkeys (ORIENTAL pellets). During the treatment period, two monkeys (#1, #3) were given LK-903 mixed in mashed bananas and one monkey (#2) a suspension of LK-903 in water with stomach tube daily for one week. Blood samples were taken from the femoral vein at weekly intervals.

Beagle dogs were purchased from Nihon CLEA Co., Ltd. (Tokyo), and maintained on the dog chow (Taiyo Pet Food Co., Ltd., Tokyo). The experimental dogs were given the drugs in capsules daily for 4 weeks and blood samples were taken weekly from the accessory cephalic vein.

1) Location: *Toda, Saitama 335, Japan.*

2) K. Takashima, K. Ohyama, T. Mori, and S. Takeyama, *Biochem. Pharmacol.*, in press.

3) S. Ohshima, H. Satoh, S. Nagase, K. Hirohashi, and H. Ohmi, *Exp. Animals*, **22**, suppl., 1973; "Proceedings of the ICLA Asian Pacific Meeting of Laboratory Animals Sept. 20—25, 1971, Tokyo and Inuyama," p. 253.

4) B. Zak, R.C. Dichenman, E.G. White, H. Burnett, and P.J. Cherney, *Am. J. Clin. Pathol.*, **24**, 1307 (1954).

LK-903 and α -methyl-*p*-myristyloxycinnamic acid (LK-903 acid) were supplied by Dr. Hayashi of the Products Formulation Research Laboratory of Tanabe Seiyaku Co., Ltd.⁵⁾

Results and Discussion

When miniature pigs were treated with LK-903 or clofibrate in the diet for one week, marked reductions of serum cholesterol were observed (Table I). The highest degree of cholesterol depression was attained at the LK-903 dose of 50 mg/kg, the higher doses (100, 300 mg/kg) being less effective by unknown reasons. Significant lowerings of serum chole-

TABLE I. Hypocholesterolemic Effects of LK-903, LK-903 Acid and Clofibrate in Miniature Pigs (OHMINI-875)

Drugs	Dose (<i>p.o.</i>) mg/kg/day for one week	Serum cholesterol ^{a)} mg/100 ml	mg/100 ml		% decrease
			Day 0	Day 7	
Control ^{b)}	(135)	78±2	71±1	9±1	
LK-903	300 (4)	62±3	37±6	40±9	
	100 (4)	72±4	38±6	46±8	
	50 (4)	58±5	13±2	79±3	
	20 (2)	73	41	45	
	10 (3)	61±7	40±10	37±12	
	5 (7)	64±3	50±3	20±6	
LK-903 acid	1 (2)	70	56	22	
	20 (2)	65	27	53	
	10 (3)	62±2	53±8	14±10	
	5 (8)	66±5	55±3	15±3	
Clofibrate	1 (3)	67±6	58±9	12±6	
	100 (4)	59±1	23±3	62±4	
	10 (8)	58±3	40±5	31±6	
	5 (4)	64±3	57±4	11±4	

a) The means±S.E.M., the number in parentheses indicates the number of experiments.

b) Cases which showed a serum cholesterol depression after a control period only due to natural fluctuation.

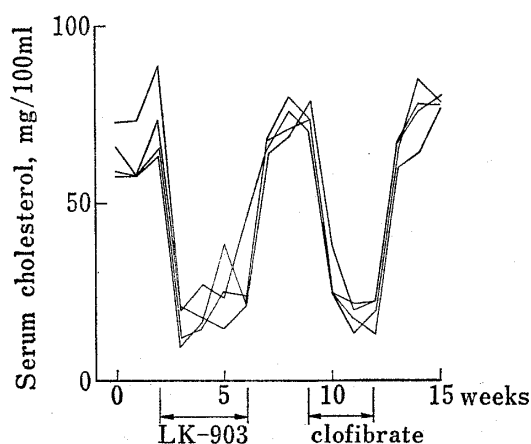


Fig. 1. Hypocholesterolemic Effect of LK-903 and Clofibrate in Miniature Pigs

LK-903, 50 mg/kg/day in diet for 5 weeks.

Clofibrate, 100 mg/kg/day in diet for 3 weeks.

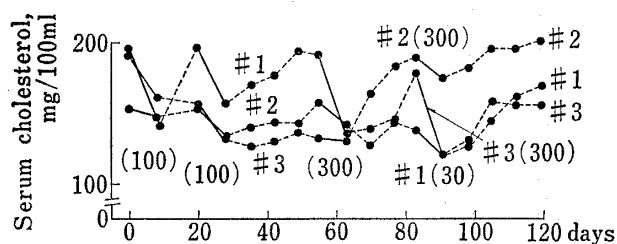


Fig. 2. Hypocholesterolemic Effect of LK-903 in Cynomolgus Monkeys

—●—: administration of LK-903 at the doses(mg/kg/day) indicated in parentheses for 7 days.

- - -○- - -: no drug.

5) T. Watanabe, T. Hashimoto, S. Yoshimatsu, S. Takeyama, K. Takashima, and K. Izumi, Japanese Patent No. 768353 (May 7, 1975); Publication No. 28743 (July 29, 1974).

terol were observed at a minimum dose of 5 mg/kg/day of LK-903 or 10 mg/kg/day of clofibrate (Table I). The minimum effective doses of LK-903 and clofibrate in male Sprague-Dawley rats were about 20 and 50 mg/kg/day, respectively.²⁾ Thus, the miniature pig is much more sensitive to the hypocholesterolemic actions of these drugs than the rat. LK-903 acid seems to be somewhat less active than LK-903 in the miniature pig as in the rat.²⁾ When high doses of LK-903 (50 mg/kg/day) and clofibrate (100 mg/kg/day) were administered, the serum cholesterol was depressed to extremely low levels (10–20 mg/100 ml) and this state of hypocholesterolemia was maintained as long as the drugs were administered (Fig. 1).

Swine are increasingly utilized as an experimental animal for studies of atherosclerosis because of some similarities between man and pigs in the progression of this disease.⁶⁾ But reports on the efficacy of hypolipidemic drugs in swine are scarce in the literature. Kim *et al.* reported that clofibrate was effective in Yorkshire swine.⁷⁾

The miniature pig OHMINI-875 is a hybrid whose genes consist of 87.5% from a Chinese wild boar and 12.5% from the Minnesota No. 1.³⁾ OHMINI-875 rarely exceeds 50 kg in body weight if maintained on restricted rations. Another advantage with this miniature pig as experimental animal is that it has large earlobes allowing repeated blood sampling from the ear vein without difficulty.

Our data showed that the sensitivity of OHMINI-875 to the hypocholesterolemic action of clofibrate is as high as in humans. The fact that LK-903 was even more active than clofibrate in the miniature pig suggests that LK-903 might be effective in humans in doses less than the therapeutic dose of clofibrate.

Efficacy of LK-903 was then examined in three cynomolgus monkeys. Their serum cholesterol levels were much higher than those of the miniature pig and, in this respect, they are similar to humans. The monkeys underwent a total of four trials of the week-long treatment with LK-903 intermittently over a period of three months. The results of weekly determinations of serum cholesterol are shown in Fig. 2. LK-903 can be considered active in the monkey at the doses of 100 and 300 mg/kg/day, their average depression rates being 16.6 and 17.0%, respectively. One trial at 30 mg/kg/day showed a slight depression of 8%.

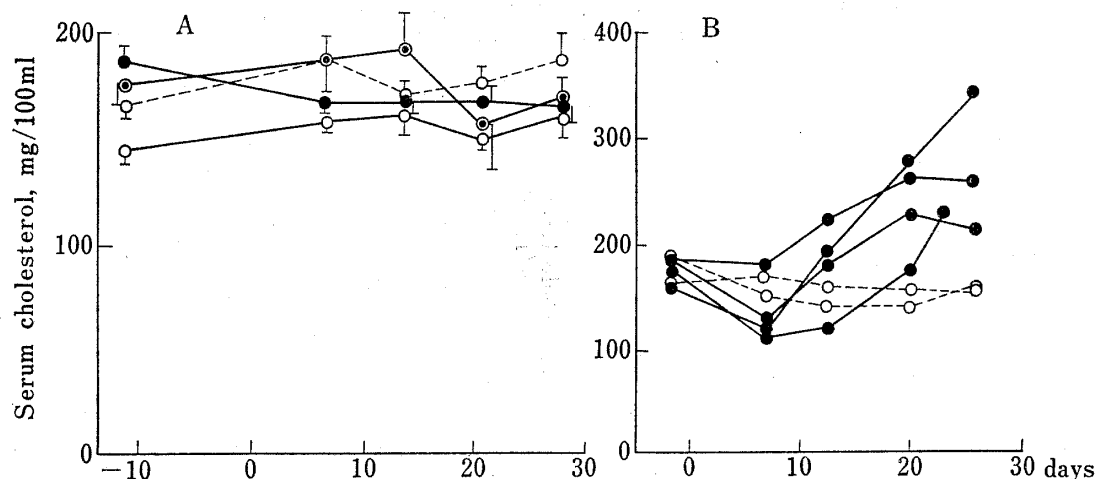


Fig. 3. Effect of LK-903 (A) and Clofibrate (B) on Serum Cholesterol in Beagle Dogs

Drugs were administered in capsules once a day from day 0. Each point with a vertical bar in A represents the mean \pm SEM of 4 or 6 dogs. Serum cholesterol levels of individual dogs are given in B. Blood samples were taken 18 hours after drug administration.

A: $\text{---}\circ\text{---}$, control; $\text{---}\circ\text{---}$, LK-903 100 mg/kg/day; $\text{---}\odot\text{---}$, LK-903 300 mg/kg/day;
 $\text{---}\bullet\text{---}$, LK-903 1000 mg/kg/day.
 B: $\text{---}\circ\text{---}$, control; $\text{---}\bullet\text{---}$, clofibrate 100 mg/kg/day.

6) H.L. Ratcliffe and H. Luginbühl, *Atherosclerosis*, **13**, 133 (1971).

7) D.N. Kim, K.T. Lee, J.M. Reiner, and W.A. Thomas, *J. Lipid Res.*, **15**, 326 (1974).

These depression rates are smaller than those obtained with the miniature pig at much lower doses (Table I). From the figure, it looks as though the monkey had bottom levels of serum cholesterol at about 130 mg/100 ml, below which there was no further lowering by LK-903. In contrast, serum cholesterol levels of the miniature pig could be brought down to as low as 10 mg/100 ml by LK-903 (Fig. 1). Another finding worthy of note with respect to the response of the monkey to LK-903 was that, once its serum cholesterol was depressed by LK-903, its recovery to the normal level was a relatively slow process taking more than a few weeks after cessation of the treatment.

In contrast to the miniature pig and the cynomolgus monkey, the beagle dog was quite resistant to the hypocholesterolemic action of LK-903. Oral administration of LK-903 in capsules to groups of 4 to 6 dogs of both sexes at the doses of 100, 300 and 1000 mg/kg/day for 4 weeks showed no cholesterol lowering effects (Fig. 3A). Oral administration of clofibrate at the dose of 100 mg/kg/day to 4 beagle dogs brought about serum cholesterol depressions of 30 to 40% after the first week of treatment in 3 of the 4 dogs, but after 3 weeks of treatment the serum cholesterol levels were higher than the starting levels (Fig. 3B) in contrast to the sustained lowering effect of clofibrate on the serum cholesterol level in the miniature pig (Fig. 1). Gans and Cater have reported a hypocholesterolemic effect of clofibrate in mongrel dogs, which were treated at the dose of 50 mg/kg/day or less for 6 or 11 days,⁸⁾ although they stated that in dogs with relatively low plasma cholesterol concentrations no changes accompanied the clofibrate treatment.

The present studies show that efficacy of a hypocholesterolemic drug can be quite variable from species to species. Although we have not tested the effect of clofibrate in the cynomolgus monkey, Thorp reported that this drug, when administered alone, was ineffective in the rhesus monkey.⁹⁾ The fact that the miniature pig, like humans, has a high sensitivity to the hypocholesterolemic action of clofibrate suggests that this species might be the animal of choice for pre-clinical studies of hypocholesterolemic agents.

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8) J.H. Gans and M.R. Cater, *Biochem. Pharmacol.*, **20**, 3321 (1971).

9) J.M. Thorp, *Lancet*, **1962**, 1323.