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Evaluation of Bioavailability of Hypolipidemic Compound LK-903[1)]

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The bioavailability of two scarcely soluble hypolipidemic compounds, 1-*O*-[*p*-(myristyloxy)-α-methylcinnamoyl]glycerol (LK-903) and its free acid (LK-A: the parent compound), was evaluated in beagle dogs by determining plasma levels by means of thin-layer chromatography (TLC) scanning densitometry.

Following oral administration of LK-903, the main chemical species in plasma were acylated forms in which natural fatty acid(s) was incorporated, while unchanged and hydrolyzed forms represented minor fractions. LK-A showed very poor bioavailability in solid formulations, but exhibited plasma levels comparable to those obtained with LK-903 in solubilized formulations.

Intravenous ingestion of LK-903 or LK-A resulted in no detectable transformation to the acylated forms, whereas administration of authentic sample of mono- or diacylated LK-903 produced considerable amounts of LK-903 and LK-A in the plasma, indicating the occurrence of hydrolysis in the systemic circulation.

On the basis of these findings, the mechanisms of intestinal absorption were supposed to resemble that of fat absorption, in which solubilization by bile acids and transacylation in the intestinal mucosa may play important roles. In relation to these absorption mechanisms, it is possible to compare the effect of chemical modification with the glyceride moiety with that of pharmaceutical modification of the dosage form for the parent compound.

Keywords——hypolipidemic compound; cinnamic acid derivative; bioavailability; absorption mechanism; dosage form; preformulation study; plasma concentration

Introduction

1-*O*-[*p*-(myristyloxy)-α-methylcinnamoyl]glycerol (LK-903) is an oral hypolipidemic compound synthesized by Watanabe *et al.*[2)] in our labovatory. It has been found that this compound possesses stronger efficacy than clofibrate in rat[3)] and miniature pig,[4)] and also in other animals. Takashima *et al.*[5)] made the interesting observation that the parent compound, *p*-(myristyloxy)-α-methylcinnamic acid (LK-A), showed less hypolipidemic activity than LK-903 regardless of its strong inhibitiory effect on the epinephrine-induced lipolysis by rat epididymal adipocytes *in vitro*.[3,4)] These data suggest that the mode of gastrointestinal absorption of LK-903 and LK-A may affect the pharmacological activity *in vivo*.

The present investigation was undertaken to clarify the biopharmaceutical characteristics of LK-903 as compared to LK-A. These compounds, as well as acylated homologues of LK-903, were administered orally or intravenously to beagle dogs in various dosage formulations and the resultant plasma levels were compared. On the basis of the bioavailability profiles of LK-903 and LK-A thus obtained, the mechanisms of intestinal absorption as well as the role of the glyceride moiety in the absorption of LK-903 are discussed.

Experimental

Materials——Animals: Beagle dogs were purchased from Yoshiki Yakko Co. and maintained on dog chow (Oriental Yeast Co.). The dogs were fasted for 18 h prior to and 8 h after administration of drugs.

Chemicals: Yolk lecithin was prepared at our laboratory.[6] Surfactants were purchased from Nikko Chemicals and Kao-Atlas Chemicals. Other chemicals were special grade reagents from Nakarai Chemicals. Porcine pancreas lipase type VI and precoated thin-layer chromatography (TLC) plates (Silica gel $60F_{254}$) were purchased from Sigma and Merck Co., respectively. LK-903 and its homologues were supplied by Watanabe *et al.* in our laboratory. The water solubility of each compound is less than 1 μg/ml. The molecular formulae are presented in Fig. 1.

Dosage Form: Lecithin Mixture: LK-903 or a homologue and egg yolk lecithin were mixed in a ratio of 1:16 (w/w). The mixture was dissolved in chloroform, then the solvent was completely removed under reduced pressure.

Lecithin Solution: The lecithin mixture was dissolved in 16 volumes of water.

Emulsion of LK-903: LK-903, Tween 80[7] and water were mixed in a ratio of 1:25:100 and emulsified by sonication for 5 min.

Oil Solution of LK-A: LK-A, HCO-60,[7] SPAN-85[7] and linoleic acid were mixed in a ratio of 3:10:2:25 (w/w) and then solubilized by heating at 80 °C in a water bath.

Oil Suspension of LK-A: LK-A was added to soybean oil (1:9) and shaken by hand. Experimental tablets containing LK-903 or LK-A were prepared by using a rotary tablet machine. Their characteristics are given in Table I.

Animal Experiments——Each preparation was administered orally by compulsive swallowing with 30 ml of water or intravenously to beagle dogs after overnight fasting unless otherwise specified. No food was given until the last blood sampling time, but water was freely supplied. Doses for oral and intravenous administration were 100 mg and 10 mg LK-903 eq, respectively. Blood samples were withdrawn at 0, 1, 2, 3, 4, 5 and 7 h after *p.o.* and 0, 3, 6, 9, 12, 15, 20, 30, 45, 60, 90, 120 and 180 min after *i.v.* administration.

Determinations of Plasma Concentration——A 1 ml plasma specimen was put into a 15 ml test tube with a stopper and extracted with 10 ml of mixed solvent consisting of *n*-hexane, chloroform and ethanol (9:0.5:0.5). The tube was centrifuged at 2500 rpm for 5 min, then the solvent layer was transferred to a 10 ml tapered tube and

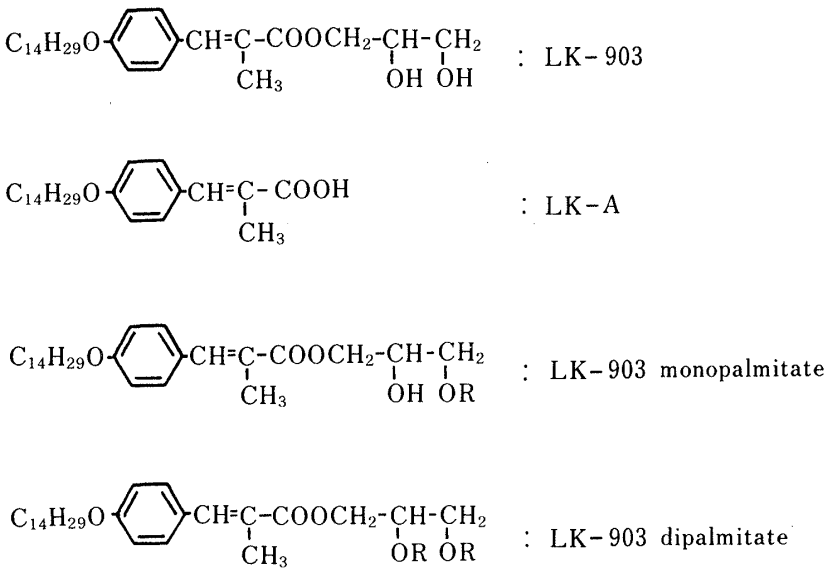

Fig. 1. Molecular Formulae of LK-903 and Its Homologues
R: $C_{15}H_{31}CO$.

TABLE I. The Pharmaceutical Characteristics of Experimental Tablets containing LK-903 or LK-A

	Tablet A	Tablet B
Active ingredient (mg)	100.0 (LK-903)	83.5 (LK-A)
Weight (mg)	250.0	249.8
Thickness (mm)	3.26	3.30
Diameter (mm)	9.0	9.0
Hardness (kg)	5.6	5.0
Disintegration time (min)	7.7	7.0

evaporated to dryness at 75 °C under a stream of nitrogen gas. The extraction and evaporation were repeated three times. The combined residues were spotted on a thin layer plate and the plate was developed for approximately 15 cm in a mixture of petroleum ether, diethyl ether and ethanol (87.5 : 12.5 : 1). The spots were scanned with a double-beam chromatoscanner (CS 910 : Shimadzu Seisakusho) and the wavelengths for sample and reference absorbances were set to 290 and 350 nm, respectively. LK-A and diacylated form of LK-903 appeared at *Rf* values of approximately 0.2 and 0.3, respectively. After being thus scanned once, the plate was redeveloped in a mixture of petroleum ether, diethyl ether, acetone and acetic acid (90 : 10 : 15 : 1). The spots of LK-903 and its monoacylated form, with *Rf* values of approximately 0.2 and 0.6, were then measured in the same way as described above.

Results and Discussion

1. Plasma Levels following Oral Administration of LK-903 and LK-A

Figure 2 shows typical densitograms obtained from plasma following oral administration of LK-903 to a beagle dog. Four peaks were detected on the thin layer chromatograms. Namely, two peaks were separated by the first development and these corresponded to LK-A

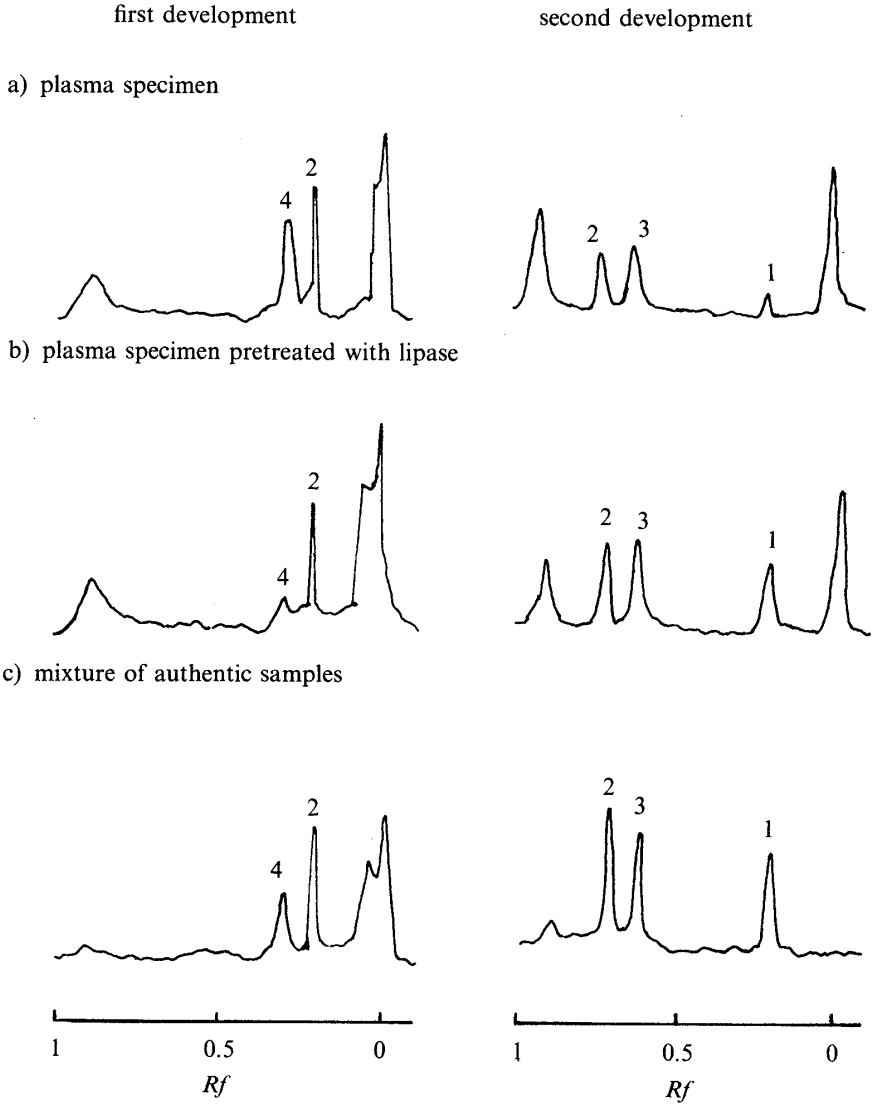

Fig. 2. TLC Densitograms of Plasma Sample following Oral Administration of LK-903

1, LK-903; 2, LK-A; 3, LK-903 monopalmitate; 4, LK-903 diplamitate.
First development; Large peaks near the origin contained 1, 2 and plasma impurities.
Second development; Peaks near the origin were plasma impurities. Peaks at the front contained plasma impurities and 4.

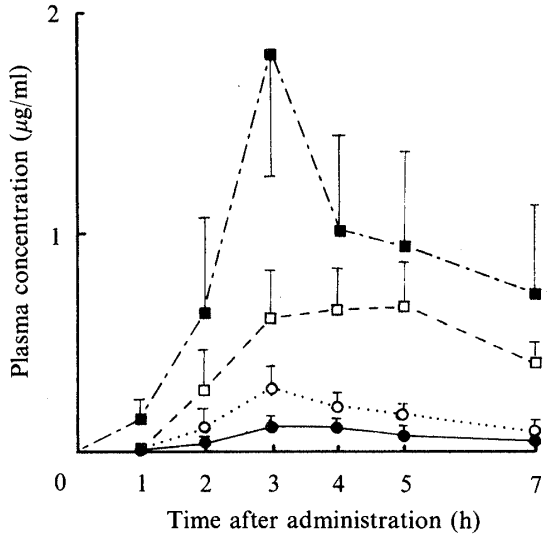

Fig. 3. Plasma Concentration Curves of LK-903 and Its Homologues following Oral Administration of LK-903 in Experimental Tablets

●, LK-903; ○, LK-A; □, monoacylated form of LK-903; ■, diacylated form of LK-903.
Each point represents the mean of four dogs.
Bars denote the standard errors.

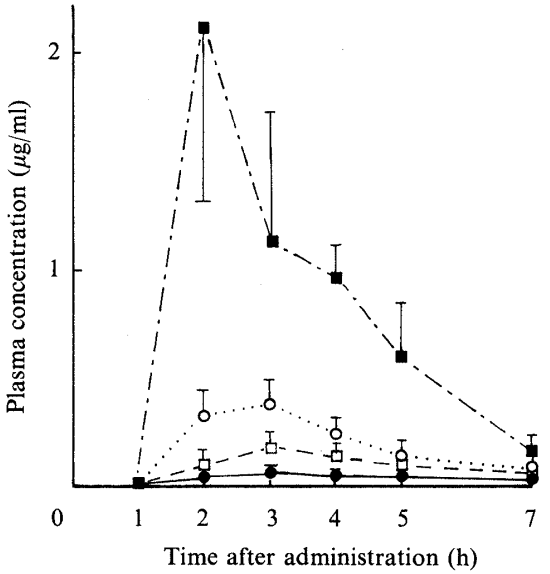

Fig. 4. Plasma Concentration Curves of LK-903 and Its Homologues following Oral Administration of LK-A in Oil Solution

●, LK-903; ○, LK-A; □, monoacylated form of LK-903; ■, diacylated form of LK-903.
Each point represents the mean of four dogs.
Bars denote the standard errors.

and LK-903 dipalmitate, respectively. Two different peaks migrated in the second development, corresponding to LK-903 and LK-903 monopalmitate, respectively. The interrelationship of these peaks was confirmed by the observation that the peak of LK-903 dipalmitate disappeared after pretreating the plasma with porcine pancreatic lipase, with simultaneous increase in the peaks of its hydrolyzed forms. These data clearly suggest that LK-903 was partially hydrolyzed to the free acid (LK-A) and partially converted to acylated forms in which natural fatty acid(s) was incorporated *in vivo*.

The plasma concentration curves of LK-903 and its three homologues after oral administration of LK-903 are shown in Fig. 3. It was apparent that the unchanged form, LK-903, and the hydrolyzed form, LK-A, were rather minor fractions and the two acylated species were major fractions; the concentration of diacylated form was about twice that of monoacylated form.

When LK-A, the parent compound of LK-903, was orally administered in capsules the plasma concentrations were too low to be detectable, indicating that LK-A shows very poor bioavailability, unlike LK-903. However, as shown in Fig. 4, oral administration of LK-A in oil solution produced detectable plasma concentrations of four homologues, as in the case of LK-903 administration. This result suggests that LK-A is absorbed well through gut mucosa in such a dosage form and its low bioavailability in the solid formulation may be attributable to its poor dispersion or emulsification in the gastrointestinal tract.

It was also found that the absorption of LK-903 was enhanced by food intake, as shown in Fig. 5. This phenomenon is consistent with the result of Takeyama *et al.*[3] that the hypolipidemic activity of a postprandial dose was stronger than that of a dose given after overnight fasting. On the other hand, the effect of food on the absorption of LK-A was obscure when LK-A was administered in an oil suspension which made it more absorbable. The enhanced absorption of LK-903 may be attributable to an increase in micellar solubilization by bile acid, as is the case for other lipophilic compounds.[8]

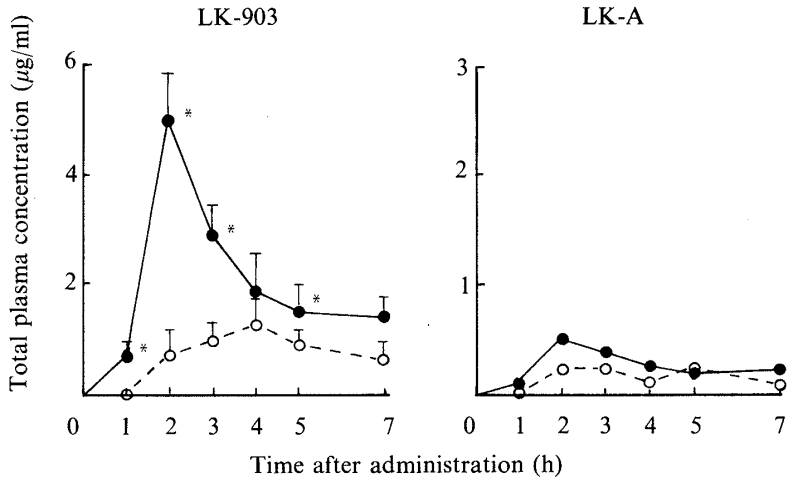

Fig. 5. Effect of Food on Bioavailability of LK-903 and LK-A

●, after feeding; ○, after overnight fasting. LK-903 and LK-A were administered in tablet A to four dogs and oil suspension to two dogs, respectively.
Each point represents the mean value.
Bars denote the standard errors.
a) Significant differences: $p<0.05$.

2. Intravenous Administration

When equimolar amounts of LK-903, LK-A, LK-903 monopalmitate and LK-903 dipalmitate were intravenously administered in lecithin solution, the plasma concentration curves shown in Fig. 6 were obtained. LK-903 and LK-A existed in only the ingested form without any conversion *in vivo*, whereas the two acylated compounds were considerably changed to hydrolyzed forms *in vivo*. It was also found that intravenous LK-903 dipalmitate gave plasma patterns of the four homologues similar to those after oral administration of LK-903 or LK-A. Another experiment, *in vitro*, showed that LK-903 dipalmitate was not hydrolyzed at all on incubation with dog blood. The difference between *in vivo* and *in vitro* metabolic conversions suggests that the compound may be hydrolyzed in the liver or it may be incorporated into the chylomicrons, where it could be readily attacked by plasma lipase *in vivo*.

The pharmacokinetic parameters evaluated according to the two compartment open model are shown in Table II. The hybrid microconstants of the distribution phase (α) were similar among the four compounds, but the hybrid microconstants of the elimination phase (β) varied considerably. The acylated derivatives were more slowly eliminated from the body than LK-903, showing longer biological half-lives ($T_{1/2\beta}$). It appeared that the acylated derivatives are readily distributed to the tissue compartment, judging from the higher ratio of the tissue distribution volume (V_d) to that of the central compartment (V_c).

3. Effect of Dosage Form on Bioavailability

The bioavailability of LK-A as well as LK-903 was compared among various oral dosage forms, such as tablets, oil suspension, lecithin mixture and lecithin solution.

Changes in the bioavailability, which were expressed in terms of the area under the total plasma concentration time curve, are summarized in Fig. 7. It was found that the bioavailability of LK-A was markedly dependent on the dosage form, being increased by more than several tens of times by the lecithin solution as compared to that of the solid dosage form. It was also found that the bioavailability of LK-903 was largely dependent on the experimental formulation. When LK-903 and LK-A were administered in the form giving the best bioavailability, *i.e.*, the lecithin solutions, they showed almost equal bioavailability. It was thus comfirmed that LK-A has an absorbability comparable with that of LK-903 if a

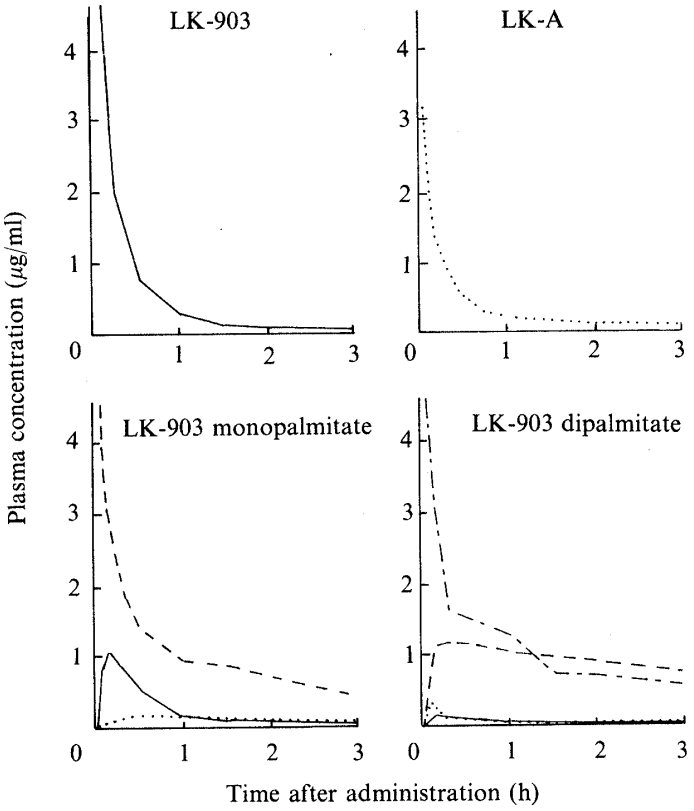

Fig. 6. Plasma Concentration Curves of LK-903 and Its Homologues following Intravenous Injection of Four LK-903 Compounds

——, LK-903; ·····, LK-A; – – –, monoacylated form of LK-903; –·–·, diacylated form of LK-903.
Each compound was injected in lecithin solution into two dogs.

TABLE II. Pharmacokinetic Parameters following Intravenous Injection of LK-903 and Its Homologues

	A (μg/ml)	B (μg/ml)	α (h^{-1})	β (h^{-1})	$T_{1/2}\beta$ (min)	V_c (liter)	V_t (liter)
LK-903	9.7	1.7	8.8	1.7	24	0.88	0.72
LK-A	3.3	0.45	6.4	0.51	81	2.7	2.9
LK-903 monopalmitate	3.2	1.4	8.8	0.36	114	2.2	3.9
LK-903 dipalmitate	7.9	1.3	8.3	0.22	184	1.1	4.7

Each compound (10 mg LK-903 eq) was intravenously injected in 2 ml of lecithin solution.
Pharmacokinetic parameters were calculated by a nonlinear least squares method with a microcomputer program. The values were obtained from the mean plasma elimination curves of two dogs.

suitable dosage form is used. This finding is consistent with the finding of Takeyama *et al.*[9] that the hypolipidemic activity of LK-A was almost the same as that of LK-903 in pharmacological examination using the lecithin solution provided by us.

It may be deduced that the glyceride moiety in the LK-903 molecule has an important role in the emulsification process with bile acids, making the parent compound readily absorbable. On the other hand, modification on the dosage form is indispensable for improvement of the bioavailability of LK-A, since it lacks this moiety.

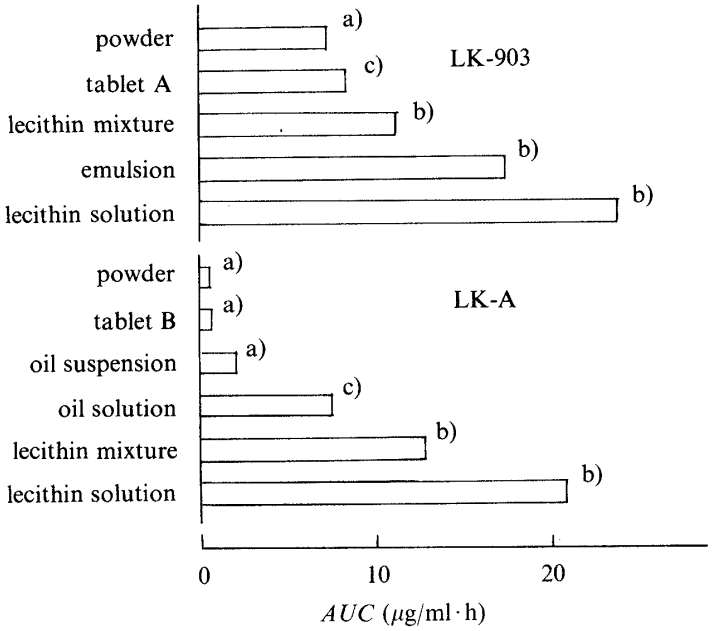

Fig. 7. Comparison of Bioavailability of LK-903 and LK-A in Various Formulations (Oral Administration)

AUC was calculated according to the trapezoidal rule by using 0—7 h total plasma concentrations.
Numbers of animals were 2 (a), 3 (b) and 4 (c).

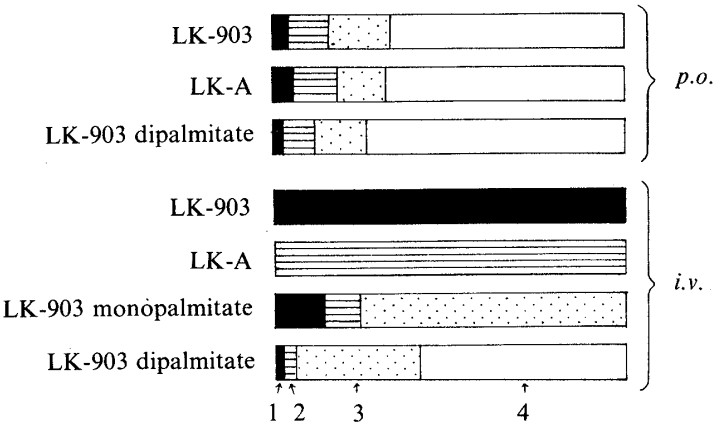

Fig. 8. Plasma Fractional Patterns of LK-903 and Its Homologues following Oral or Intravenous Administration of Four LK-903 Compounds

1, LK-903; 2, LK-A; 3, monoacylated form of LK-903; 4, diacylated form of LK-903.

4. Absorption Mechanisms of LK-903 and LK-A

Oral administration of LK-903 dipalmitate was carried out to clarify the absorption mechanisms of LK-903 and LK-A. It was found that this compound was also well absorbed and appeared in plasma as the same four homologues as were seen after oral administration of LK-903. Combining this result with the above observations, the fractional patterns are summarized in Fig. 8. The pattern after oral administration of LK-903 was completely different from that after intravenous administration, but essentially the same as that after intravenous administration of LK-903 dipalmitate. It is thus suggested that LK-903 was transported to the systemic circulation after conversion to diacylated LK-903 during the absorption process. On the basis of the oral and intravenous bioavailability profiles, the absorption mechanisms may be as shown in Fig. 9.

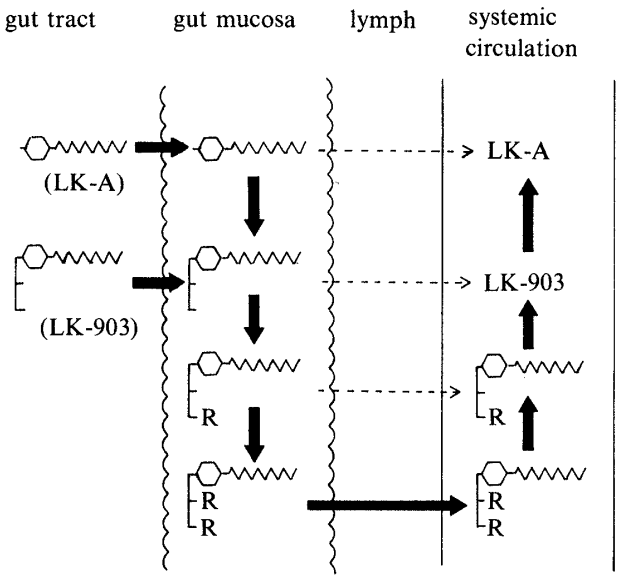

Fig. 9. Proposed Mechanisms for Absorption of LK-903 and LK-A
R: long chain fatty acyl group(s).

Namely LK-903, a monoglyceride analogue, penetrates into the gut mucosa and is then converted to the corresponding triglyceride by the well-known transacylation system[10] involved in fat absorption. The resultant triglyceride can be successively hydrolyzed and converted to LK-903 monoacylate, LK-903 and LK-A in the systemic circulation. This similarity of the absorption process of LK-903 and that of natural monoglyceride was supported by the finding of Aso *et al.*[11] that LK-903 was predominantly absorbed *via* the lymphatic duct. Furthermore the metabolic conversion of LK-A to the same homologues as in the case of LK-903 leads to the conclusion that LK-A can be absorbed in a manner similar to that of natural fatty acid.

The above findings may give some insight into the design and preformulation study of new drugs having structures analogous to those of natural fatty acid glycerides.

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References and Notes

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