

Lymphatic Absorption of Hypolipidemic Compound, 1-*O*-[*p*-(Myristyloxy)- α -Methylcinnamoyl] Glycerol (LK-903)

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The intestinal absorption process of 1-*O*-[*p*-(myristyloxy)- α -methylcinnamoyl] glycerol (LK-903), a new hypolipidemic compound, was studied in rats.

When ^3H -LK-903 or ^3H -LKA [^3H -*p*-(myristyloxy)- α -methyl cinnamic acid], labeled at the cinnamic acid moiety, or ^{14}C -LK-903, labeled at the glycerol moiety, were administered orally to thoracic duct-cannulated rats at a dose of 0.233 mmol/kg, 31.1, 6.7 and 18.1% of the dose, respectively, appeared in the lymph within 24 h. In this case, radioactive compounds in the lymph lipids consisted of LKA (radioactivity was not detected in the fraction of LKA with ^{14}C -LK-903), LK-903, diglyceride analogues and triglyceride analogues. The percentages of the triglyceride analogues were the highest, followed by the diglyceride analogues. On the other hand, when doubly labeled LK-903 ($^3\text{H}/^{14}\text{C} = 1$, corrected ratio) was administered orally, the values of $^3\text{H}/^{14}\text{C}$ for the monoglyceride, diglyceride and triglyceride analogues in the lymph were 1.2–1.5, 1.7–1.9 and 1.9–2.7, respectively. The lymphatic absorption of LK-903 was stimulated by the presence of lecithin but inhibited by a high dose of triolein. The results indicated that (1) LK-903 formed micelles in the intestine, (2) a large part of LK-903 was absorbed as such, (3) a part of LK-903 was hydrolyzed in the intestinal mucosa, and (4) a part of LKA formed by hydrolysis was again utilized to synthesize the higher glycerides and absorbed *via* the lymphatic absorption route for lipids.

Keywords — lymphatic absorption; hypolipidemic compound; acylglycerols; LK-903

Introduction

1-*O*-[*p*-(Myristyloxy)- α -methylcinnamoyl] glycerol (LK-903), α -monoacylglycerol analog, has been found to possess a potent hypolipidemic activity in experimental animals by Takashima *et al.*^{1,2)} It was observed that LK-903 was absorbed slowly *via* the lymphatic system^{3,4)} and distributed in high concentrations in the liver and adipose tissues.³⁾

Reports of the lymphatic transport of drugs and other xenobiotics are scant and only few compounds such as 3-methylcholanthrene,⁵⁾ asarone,⁶⁾ DDT⁷⁾ and naftifine⁸⁾ have been reported to be absorbed *via* the lymphatic route.

In the present study, we investigated the lymphatic absorption process of LK-903 using thoracic duct-cannulated rats.

Materials and Methods

Labeled Compounds — ^{14}C -, ^3H -LK-903 and ^3H -*p*-(myristyloxy)- α -methylcinnamic Acid (^3H -LKA): [1,3- ^{14}C]-glycerol (40 mCi/mmol) and tritium gas (2 Ci) were obtained

from New England Nuclea (NEN) Research Products. ^{14}C -LK-903 was synthesized from [1,3- ^{14}C]-glycerol, and ^3H -LK-903 and ^3H -LKA were prepared by continuous catalytic hydrogenation of 3-bromo-4-myristyloxy benzaldehyde diethylacetal (IV) with tritium gas,⁹⁾ followed by the synthetic steps shown in Fig. 1.¹⁰⁾ The specific activities of ^{14}C -, ^3H -LK-903 and ^3H -LKA were 1.02, 315 and 377 $\mu\text{Ci}/\text{mg}$, respectively, and their radiochemical purities were more than 99% as determined by thin-layer chromatography (TLC) using *n*-heptane–ether–acetic acid–methanol (85:15:2:3, v/v)¹¹⁾ as a developing solvent.

Monopalmitate and Dipalmitate Derivatives of ^3H -LK-903: These were synthesized from ^3H -LK-903 as shown in Fig. 2.¹²⁾ The specific activities were 251 and 176 $\mu\text{Ci}/\text{mg}$, respectively, and the radiochemical purities were more than 98% as determined by TLC.

Animals and Administration — Male Wistar rats (10 weeks old, weighing about 350 g) were used in the experiments of lymphatic absorption and rats (7 weeks old, weighing about 200 g) were used for other experiments.

$$\begin{array}{c}
 \text{HO}^{14}\text{CH}_2\text{CH}^{14}\text{CH}_2 + n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)-\text{COCl} \xrightarrow{\text{pyridine}} n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)-\text{CO}_2^{14}\text{CH}_2\text{CH}^{14}\text{CH}_2 \\
 \text{I} \qquad \qquad \qquad \text{II} \qquad \qquad \qquad \text{III} \\
 \downarrow \text{H}^+ \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)-\text{CO}_2\text{CH}_2\text{CH}^{14}\text{CH}_2\text{OH} \\
 \text{}^{14}\text{C-LK-903}
 \end{array}$$

$$\begin{array}{c}
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(\text{Br})-\text{CH}(\text{OEt})_2 \xrightarrow[10\% \text{ Pd-C}]{^3\text{H}_2 \text{ gas}} n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CH}(\text{OEt})_2 \xrightarrow{\text{H}^+} n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CHO} \\
 \text{IV} \qquad \qquad \qquad \text{V} \qquad \qquad \qquad \text{VI} \\
 \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CH}=\text{C}(\text{CH}_3)\text{COOH} \rightarrow n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CH}=\text{C}(\text{CH}_3)\text{COCl} \xrightarrow{\text{HOCH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{OH}} \\
 ^3\text{H-LKA} \qquad \qquad \qquad \text{VIII} \\
 \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{OH} \xrightarrow{\text{H}^+} n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_3(^3\text{H})-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH} \\
 \text{IX} \qquad \qquad \qquad ^3\text{H-LK-903}
 \end{array}$$

$$\begin{array}{c}
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH} + \text{CH}_3(\text{CH}_2)_{14}\text{COCl} \\
 \text{}^3\text{H-LK-903}
 \end{array}
 \xrightarrow{(\text{Et})_3\text{N}}
 \begin{array}{c}
 \begin{array}{c}
 \text{CH}_3(\text{CH}_2)_{14}\text{CO}_2\text{CH}_2 \\
 | \\
 \text{CHOH} \\
 | \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_2 \\
 | \\
 \text{}^3\text{H}
 \end{array} \\
 \text{or} \\
 \begin{array}{c}
 \text{HOCH}_2 \\
 | \\
 \text{CHO}_2\text{C}(\text{CH}_2)_{14} \\
 | \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_2 \\
 | \\
 \text{}^3\text{H}
 \end{array} \\
 \text{monopalmitate of } ^3\text{H-LK-903} \\
 \text{or} \\
 \begin{array}{c}
 \text{CH}_3 \\
 | \\
 n\text{-C}_{14}\text{H}_{29}\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{C}(\text{CH}_3)\text{CO}_2\text{CH}_2 \\
 | \\
 \text{}^3\text{H}
 \end{array} \\
 \text{dipalmitate of } ^3\text{H-LK-903}
 \end{array}$$

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Each labeled compound was appropriately diluted with the non-labeled compound and suspended in a 0.1% Nikkol HCO60 (hydrogenated castor oil) solution with a sonicator (Tomy UR 150P) for oral administration at a dose of 0.233 mmol/10 ml/kg.

Determination of Radioactivity — Each liquid sample (0.2 ml) was dissolved in 15 ml of scintillation fluid, which was prepared by dissolving 100 g of naphthalene, 5 g of DPO and 0.3 g of dimethyl-POPOP in a mixture of 730 ml of dioxane, 135 ml of toluene and 35 ml of methanol. Radioactivity was counted with an Aloka LSC-502 liquid scintillation spectrometer. Radioactive areas on TLC plates were scraped into counting vials and extracted with 1 ml of 20% methanol-ether (v/v). The dioxane scintillation fluid (15 ml) was added to the vial and radioactivity was measured.

Measurement of the Lymphatic Absorption Rate — The thoracic duct was cannulated according to Bollman's method with minor modifications.¹³⁾ The cannulated rats were individually housed in Bollman cages for one night. The rats, which showed normal rates of lymph flow (more than 30 ml/night), were used.

TLC¹¹⁾ — Radioactive compounds in the lymph lipids were extracted twice with 20 volumes of ethanol-ether (3:1, v/v). The extract (98% of total radioactivity in the lymph) was concentrated to a small volume under reduced pressure and then dissolved in a small volume of hexane and used for analysis by TLC. Just before use, the thin-layer plate (Merck: Kieselgel 60F₂₅₄, 0.25 mm thickness) was impregnated with acetic acid by immersing it in 2.5% acetic acid-ether for 5 min and air-dried. The extract dissolved in hexane was applied to the plate and developed with a solvent system of ether-*n*-heptane-acetic acid-methanol (15 : 85 : 2 : 3, v/v). The plate was placed in contact with Sakura Industrial X-ray film (type N) for few days. The film was developed with a developer (Konidol X) to give a TLC-autoradiogram. Radioactivity on the plate was measured as described above.

Lymphatic Absorption of Single-Labeled LKA, LK-903 and Double-Labeled LK-903 — Each 0.233 mmol/kg of ¹⁴C-LK-903 (1–2

μCi/rat), ³H-LK-903 (10–20 μCi/rat), ³H-LKA (10–20 μCi/rat) and ³H, ¹⁴C-LK-903 (³H/¹⁴C = 4.9/1) was administered orally to the thoracic duct-cannulated rats and the lymph was collected for intervals of 0–2, 2–4, 4–6 and 6–24 h. Aliquots were used for determination of radioactivity and for analysis of radioactive compounds by TLC.

Effects of Vehicles on Lymphatic Absorption of ³H-LKA and ³H-LK-903 — Triolein: Emulsions of triolein, 500 mg or 3 g, and ³H-LK-903, 100 mg (0.233 mmol), in 10 ml of a 0.1% Nikkol HCO 60 solution were prepared with a sonicator and 3.5 ml of the emulsion were administered orally to the thoracic duct-cannulated rats.

Lecithin: Emulsions of ³H-LK-903 100 mg or ³H-LKA 83.5 mg (both 0.233 mmol) and 170 mg of lecithin in 10 ml of distilled water were prepared with a sonicator and 3.5 ml of the emulsion were administered orally to the thoracic duct-cannulated rats.

Hydrolysis of LK-903 and Its Related Compounds by Lipase — Porcine pancreas lipase (type II, 47 units/mg protein) was purchased from Sigma Chemical Company. Mono- or dipalmitate of ³H-LK-903 was dissolved in 0.05 ml of ethanol and then suspended in 0.5 ml of a 1% Arabia gum solution. Enzyme (9 mg) was weighed into a 10 ml test tube and dissolved in 0.6 ml of 0.2 M potassium phosphate buffer (pH 7.4). After preincubation of the enzyme solution for 5 min in a water bath at 37 °C, 0.2 ml of 5 mM mono or dipalmitate of ³H-LK-903 was added and incubated further for 30 or 60 min. At the end of the incubation period, 2.5 ml of a Dole's extract solution (isopropyl alcohol-*n*-heptane-1 N sulfuric acid = 40:10:1, v/v)¹⁴⁾ containing 8×10^{-5} M of non-labeled LKA was added to stop the lipolysis. The lipids were extracted twice with 2.5 ml of a Dole's extract solution. The extract was concentrated to dryness under vacuum and then dissolved in a small volume of hexane and analyzed by TLC.

Results

Lymphatic Absorption of Single-Labeled LKA and LK-903

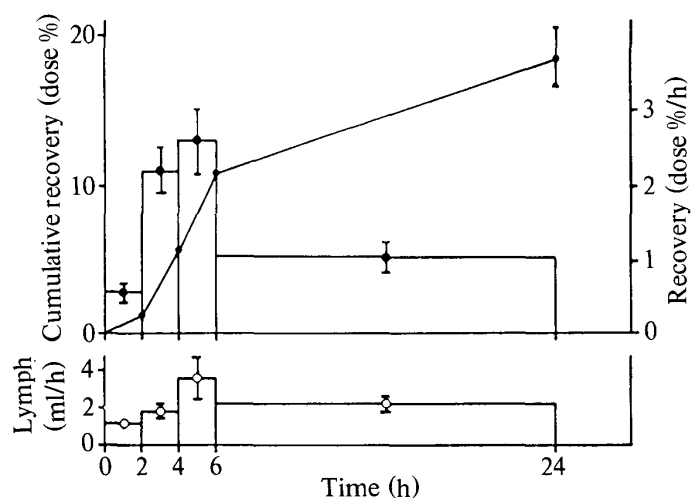


Fig. 3. Recovery of Radioactivity and Volume of Lymph (ml/h) at 2, 4, 6, 24 h after Oral Administration of ^{14}C -LK-903 to Rats (100 mg/kg)
Each point represents the mean \pm S.E. of 3 animals.

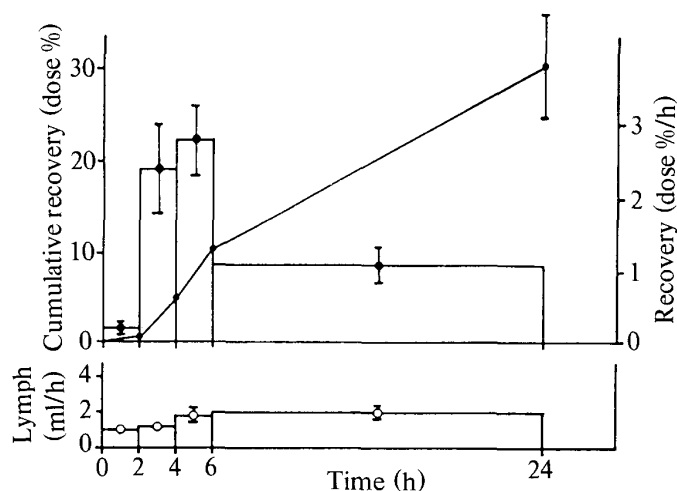


Fig. 4. Recovery of Radioactivity and Volume of Lymph (ml/h) at 2, 4, 6, 24 h after Oral Administration of ^3H -LK-903 to Rats (100 mg/kg)
Each point represents the mean \pm S.E. of 3 animals.

Time courses of the rates of lymphatic absorption of ^{14}C -LK-903 and ^3H -LK-903 are shown in Figs. 3 and 4. When ^{14}C -LK-903 or ^3H -LK-903 was administered orally to rats, 18.1% or 31.1% of radioactivity of the dose appeared in the thoracic duct lymph within 24 h, respectively. The maximum absorption rates were observed within 4–6 h with both labeled compounds, and these values were 2.6% of the dose/h with ^{14}C -LK-903 and 2.8% of the dose/h with ^3H -LK-903.

After oral administration of ^3H -LK-903 or ^{14}C -LK-903 the radioactive compounds in the lymph were separated into the fractions of phospholipides, monoglycerides, free fatty acids, diglycerides and triglycerides using TLC as described by Belfrage *et al.*¹¹⁾ Their TLC-autoradiograms closely resembled each other except that radioactivity was detected in the area of LKA with ^3H -LK-903 but not with ^{14}C -LK-903. Although the TLC-autoradiogram obtained with ^3H -LK-903 was not as clear as that with ^{14}C -LK-903, a typical TLC-autoradiogram of 4–6 h lymph extracts obtained after oral administration of ^{14}C -LK-903 is shown in Fig. 5. The TLC-autoradiogram showed seven radioactive bands. Band 1 appeared to be phospholipids in view of the results reported by Belfrage *et*

*al.*¹¹⁾ who studied the appearance of radioactivity in partial glycerides of rat livers after administration of the triglyceride labeled with ^{14}C -glycerol and ^3H -palmitic acid by TLC. Bands 2 and 3 are the monoglyceride analogues, because their *R_f* values corresponded to those of unchanged LK-903 and the *cis* isomer of LK-903, respectively. Bands 4 and 5 corresponded to those of β - and α -monopalmitate derivatives of LK-903, respectively, and thus, bands 4, 5 and 6 should be the diglyceride analogues in which one fatty acid was incorporated into the glycerol moiety of LK-903 or *cis* LK-903. Band 7 should be triglyceride analogues in which two fatty acids were incorporated into the glycerol moiety of LK-903, because the *R_f* value of the band 7 was similar to that of the dipalmitate derivative of LK-903 (a triglyceride analogue).

Tables I and II show the distribution of radioactivity among the glycerides in the lymph after oral administration of ^{14}C -LK-903 or ^3H -LK-903. After administration of ^{14}C -LK-903, the level of radioactivity in the triglyceride fraction was the highest, 67.6–74.4% of the total radioactivity in the lymph, and that of LK-903 and its isomer was only 8.9–14%. After administration of ^3H -LK-903, the level of radioactivity in the

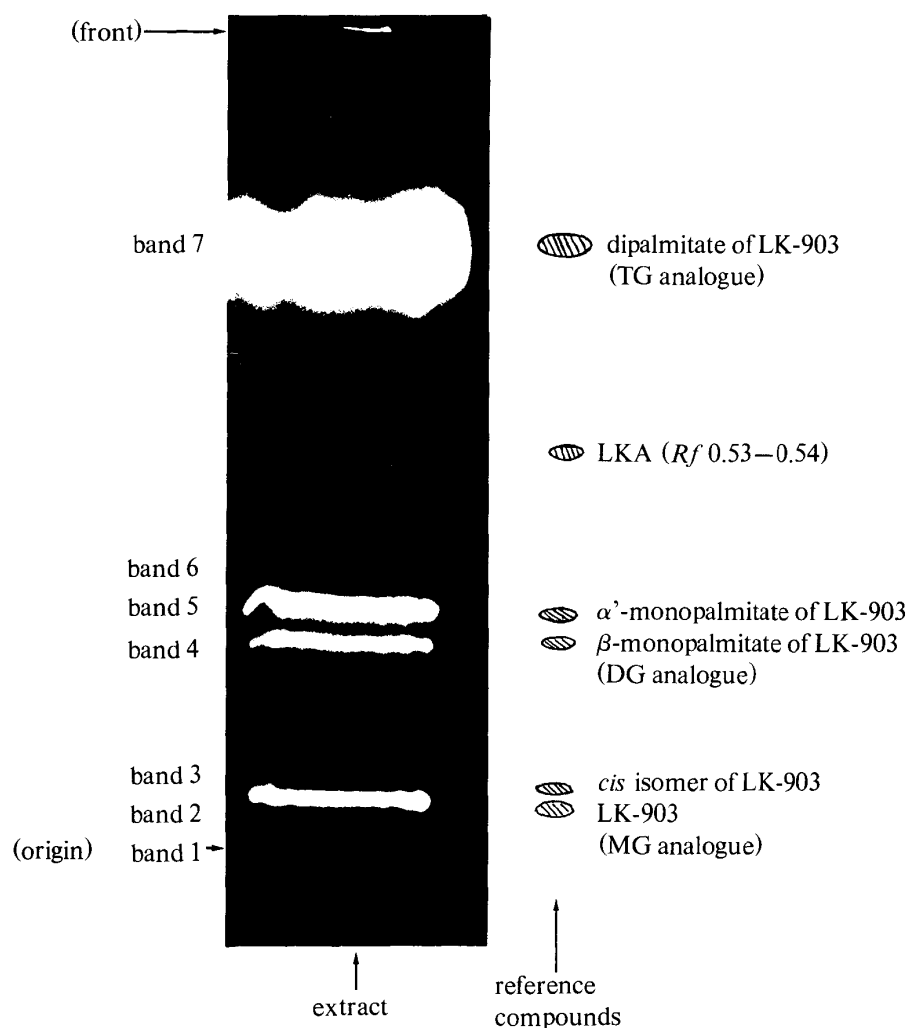


Fig. 5. TLC-Autoradiogram of Labeled Compounds in 4–6 h Lymph after Oral Administration of ^{14}C -LK-903 to Rats (100 mg/kg)

Solvent system: *n*-heptane-ether-AcOH-MeOH (85:15:2:3, v/v).

MG, monoglyceride; DG, diglyceride; TG, triglyceride.

TABLE I. Distribution of ^{14}C Radioactivity among the Glycerides in the Lymph after Oral Administration of ^{14}C -LK-903 to Rats (100 mg/kg)

Time (h)	% total ^{14}C -radioactivity in lymph lipids		
	LK-903 (Monoglyceride analogue)	Diglyceride analogues	Triglyceride analogues
0–2	8.9 ± 1.4	16.7 ± 1.1	74.4 ± 2.3
2–4	10.4 ± 1.3	17.8 ± 1.6	71.8 ± 2.6
4–6	14.0 ± 3.0	18.4 ± 0.7	67.6 ± 2.6
6–24	11.1 ± 1.5	19.4 ± 3.7	69.5 ± 5.0

Each value represents the mean \pm S.E. of 3 animals.

triglyceride fraction was also the highest in each period. The amount of LK-903 was 15.3% for 0–2 h and decreased gradually for 6 h (4.4%). LKA accounted for 8 to 10% within 4 h after ad-

TABLE II. Distribution of ^3H Radioactivity among the LK-903 Metabolites in the Lymph after Oral Administration of ^3H -LK-903 to Rats (100 mg/kg)

Time (h)	% total ^3H -radioactivity in lymph lipids			
	LK-903 (Monoglyceride analogue)	Diglyceride analogues	LKA	Triglyceride analogues
0— 2	15.3 \pm 0.8	27.0 \pm 1.6	8.4 \pm 0.9	49.3 \pm 1.0
2— 4	8.8 \pm 2.7	18.0 \pm 6.2	9.8 \pm 3.0	63.4 \pm 5.9
4— 6	4.4 \pm 1.1	11.6 \pm 1.1	5.8 \pm 2.3	78.2 \pm 3.7
6—24	8.7 \pm 1.2	19.3 \pm 2.3	3.6 \pm 1.5	68.4 \pm 3.7

Each value represents the mean \pm S.E. of 3 animals.

TABLE III. Distribution of ^3H Radioactivity among the LKA Metabolites in the Lymph after Oral Administration of ^3H -LKA to Rats (83.5 mg/kg)

Time (h)	% total ^3H -radioactivity in lymph lipids			
	LK-903 (Monoglyceride analogue)	Diglyceride analogues	LKA	Triglyceride analogues
0— 2	3.8 \pm 0.1	18.7 \pm 5.1	11.7 \pm 5.0	66.0 \pm 10.7
2— 4	3.8 \pm 0.5	12.6 \pm 1.4	2.9 \pm 0.2	80.5 \pm 1.8
4— 6	5.2 \pm 0.7	16.5 \pm 1.1	4.0 \pm 0.9	74.5 \pm 1.1
6—24	8.2 \pm 1.6	18.1 \pm 2.7	12.5 \pm 7.3	61.3 \pm 8.5

Each value represents the mean \pm S.E. of 3 animals.

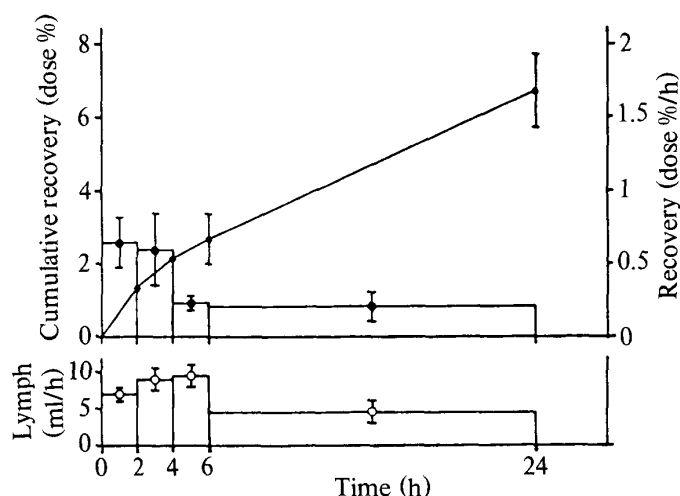


Fig. 6. Recovery of Radioactivity and Volume of Lymph (ml/h) at 2, 4, 6, 24 h after Oral Administration of ^3H -LKA to Rats (83.5 mg/kg)

Each point represents the mean \pm S.E. of 3 animals.

ministration.

When ^3H -LKA was administered, 6.7% of the dose appeared in the lymph within 24 h. The maximal rate of absorption was 1.2% of the dose/h, for the first 2 h (Fig. 6). The change in percentage of radioactivity among various levels of glyceride formation with time is shown in Table III. The triglyceride fraction represented the highest concentration, 61.3–80.5%, followed by the diglyceride fraction (12.6–18.7%). The α -monoglyceride fraction (LK-903) and LKA showed about the same concentration in each period except the 2 h period (3.8 and 11.7%, respectively).

Lymphatic Absorption of Double-Labeled LK-903

After administration of double-labeled LK-903 to rats, about 15% of the given ^{14}C and about 35% of the given ^3H were found in the 24 h-lymph. The absorption patterns were similar

TABLE IV. Distribution of Radioactivity and Ratios of $^3\text{H}/^{14}\text{C}$ in the Lymph after Oral Administration of ^{14}C -, ^3H -LK-903 (100 mg/kg)

Time (h)	LK-903 (Monoglyceride analogue)		Diglyceride analogues		LKA		Triglyceride analogues	
	% of ^3H	$^3\text{H}/^{14}\text{C}$	% of ^3H	$^3\text{H}/^{14}\text{C}$	% of ^3H	$^3\text{H}/^{14}\text{C}$	% of ^3H	$^3\text{H}/^{14}\text{C}$
0— 2	12.1±2.6	1.4	16.1±4.2	1.7	6.1±2.4	—	65.7±8.9	1.9
2— 4	9.0±1.6	1.5	14.6±1.0	1.9	3.5±0.7	—	72.9±2.6	2.4
4— 6	8.6±1.1	1.5	13.3±1.1	1.8	2.4±0.6	—	75.7±1.3	2.5
6—24	6.1±2.0	1.2	14.2±2.7	1.9	3.8±0.7	—	75.9±6.0	2.7

Values expressed as percent of total ^3H radioactivity in the chyle lipids and as ratios of $^3\text{H}/^{14}\text{C}$ relative to the ratio $^3\text{H}/^{14}\text{C}$ of ^{14}C -, ^3H -LK-903 administered.

Each value represents the mean \pm S.E. of 4 animals.

TABLE V. Lipolysis of LK-903 and Its Related Compounds

Products	Substrates					
	LK-903		Diglyceride analogues		Triglyceride analogues	
	30	60 (min)	30	60 (min)	30	60 (min)
LK-903	100	100	65.0	72.4	5.6	6.3 (%)
Diglyceride analogue	0	0	35.0	27.6	48.3	48.8
LKA	0	0	0	0	0	0
Triglyceride analogue	0	0	0	0	46.1	44.9

Each substrate was reacted with lipase in a phosphate buffer solution (pH 7.4) at 37 °C.

Lipase, 9 mg lipase from hog pancreas (47 units/mg); substrates, 1 μmol of LK-903, diglyceride and triglyceride analogue; diglyceride analogue, monopalmitate of LK-903; triglyceride analogue, dipalmitate of LK-903.

Each value represents the mean of 3 experiments.

to those after administration of each single-labeled LK-903. The ratio of $^3\text{H}/^{14}\text{C}$ of the triglyceride fraction was the highest, 1.9–2.7 times the ratio of $^3\text{H}/^{14}\text{C}$ of the administered double-labeled LK-903, followed by the diglyceride fraction and finally the monoglyceride fractions (Table IV).

Hydrolysis of LK-903 and Its Reference Compounds by Pancreatic Lipase

Since ^3H -LK-903 was found to be partially hydrolyzed in the gut after oral administration, we investigated whether LK-903, the diglyceride and the triglyceride analogues are hydrolyzed by the pancreatic lipase in the gut.

LK-903 was not hydrolyzed by pancreatic lipase *in vitro* but the diglyceride analogues com-

posed of the α - and β -monopalmitate of LK-903 were hydrolyzed by the same enzyme. After 1 h incubation, about 72.4% of the diglyceride analogues disappeared and were converted to LK-903, but LKA was not produced. During the same incubation time, the dipalmitate (triglyceride analogue) of LK-903 was hydrolyzed to produce the diglyceride analogues and LK-903 by 48.8 and 6.3%, respectively (Table V).

Effect of Vehicles on Lymphatic Absorption of LK-903 and LKA

The effect of various vehicles on the lymphatic absorption rates of ^3H -LK-903 and ^3H -LKA in various vehicles were determined.

After oral administration of an emulsion of

TABLE VI. Effect of Vehicles on the Lymphatic Absorption of LKA and LK-903

Compounds (Dose)	Dosage form	Percent of the dose absorbed <i>via</i> lymphatics within 24 h
³ H-LKA (83.5 mg/kg)	0.1% Nikkol (10 ml/kg)	6.7 ± 1.0 (<i>n</i> = 3)
	Lecithin (170 mg/kg)	47.9 ± 6.4 (<i>n</i> = 4)
³ H-LK-903 (100 mg/kg)	0.1% Nikkol (10 ml/kg)	31.1 ± 5.8 (<i>n</i> = 3)
	0.1% Nikkol + triolein (500 mg/kg)	20.8 ± 4.2 (<i>n</i> = 3)
	0.1% Nikkol + triolein (3 g/kg)	21.7 (<i>n</i> = 2)
	Lecithin (170 mg/kg)	41.4 ± 3.5 (<i>n</i> = 4)

Each value represents the mean or mean ± S.E.

100 mg ³H-LK-903 in triolein 500 mg or 3 g and 10 ml/kg of a 0.1% Nikkol solution, the lymphatic absorption rates of ³H-LK-903 were 20.8 and 21.7% of the dose, respectively, and 31.1% after oral administration of ³H-LK-903 alone in 0.1% Nikkol (Table VI). When ³H-LK-903 was orally administered as an emulsion in a lecithin (170 mg/10 ml/kg) solution, the absorption rate of ³H-LK-903 was higher (41.4%) than that after oral administration of ³H-LK-903 in 0.1% Nikkol. Similarly, when ³H-LKA was orally administered with lecithin, the lymphatic absorption rate (47.9%) was about 7 times of that (6.7%) after oral administration of ³H-LKA with Nikkol (Table VI).

Discussion

The lymphatic absorption rate of ¹⁴C-LK-903 labeled at the glycerol moiety was 18.1% of the dose in 24 h and that of ³H-LK-903 labeled at the cinnamic acid moiety was 31.1% (Figs. 3 and 4). From these results, it was concluded that the ³H-LKA formed by hydrolysis of ³H-LK-903 was absorbed *via* the lymphatic system, but the free ¹⁴C-glycerol formed by hydrolysis of ¹⁴C-LK-903 was not absorbed by this route. In fact, when ³H-LKA was administered to rats, 6.7% of the dose was recovered in the lymph in 24 h (Fig. 6). Gidez *et al.*^{15,16)} and Borgström¹⁷⁾ have also reported that the liberated glycerol from glyceride was hardly absorbed *via* the lymphatic system.

The TLC-autoradiogram of radioactive compounds in the lymph after oral administration of

¹⁴C-LK-903 revealed two bands (2 and 3) of the monoglyceride fraction, as shown in Fig. 5. Band 2 was LK-903 and the other band was regarded as an isomer of LK-903. Since LK-903 is the *trans* form and an α -monoglyceride analogue, presumably band 3 is either the *cis* form or the β -monoglyceride analogue of LK-903. The *R_f* value of band 3 corresponded to that of an authentic sample of the *cis* isomer of LK-903, but not to an authentic sample of the β -monoglyceride analogue. Therefore, band 3 was attributed to the *cis* isomer of LK-903. The radioactive compounds in the lymph collected from rats administered ³H-LK-903 consisted of LKA, LK-903, diglyceride analogues and triglyceride analogues. Also Aso *et al.*⁴⁾ have found that LK-903 and its higher glyceride analogues appeared in the chylomicrone after administration of the labeled LK-903. Therefore, it is considered that LK-903 and LKA can penetrate the intestinal mucosa and be further incorporated into higher glyceride analogues and absorbed *via* the intestinal absorption route of fat or free fatty acids.

Mattson *et al.*,¹⁸⁾ Borgstrom *et al.*¹⁹⁾ and Savary *et al.*²⁰⁾ have found that fatty acids bound at the α - and α' -position of the triglycerides were hydrolyzed rapidly by pancreatic lipase while fatty acids at the β -position were hardly hydrolyzed. In the present investigation the ester bond at the α -position of the glycerol moiety of LK-903 was not hydrolyzed, but the ester bond at the β -position of the triglyceride analogues of LK-903 was hydrolyzed by pancreatic lipase *in vitro*. This may be due to the

possibility that (1) the α -methylcinnamic acid moiety of LK-903 have steric hindrance against the pancreatic lipase action, and (2) isomerization of fatty acid at the β -position to α -position of the glycerol moiety occur and the ester bond on the α -position is hydrolyzed by the pancreatic lipase.

In the study on the mechanism of intestinal absorption of monoglycerides, Johnston *et al.*^{21,22)} found that the ratio of $^3\text{H}/^{14}\text{C}$ of α -, α' -diglyceride or triglyceride synthesized by cell-free suspensions of intestinal mucosa was very similar to that of the original doubly labeled α -monoglyceride composed of ^{14}C -glycerol and ^3H -oleic acid, indicating that the intact α -monoglyceride could be directly converted into higher glycerides. In our experiments, when doubly labeled LK-903 ($^3\text{H}/^{14}\text{C} = 1$, corrected ratio) was administered orally, the values of $^3\text{H}/^{14}\text{C}$ of the monoglyceride, diglyceride and triglyceride analogues in the lymph were 1.2–1.5, 1.7–1.9 and 1.9–2.7, respectively (Table IV). These results indicated that (1) a large part of LK-903 was absorbed as such, (2) some doubly labeled LK-903 was hydrolyzed in the intestinal mucosa and (3) some ^3H -LKA formed by hydrolysis was again utilized to synthesize the higher glycerides. Since LK-903 was not hydrolyzed by pancreatic lipase, LK-903 in the gastrointestinal tract may be hydrolyzed by other enzymes, such as aliphatic esterases or an intestinal lipase reported by Senior *et al.*²³⁾

Lecithin significantly increased the lymphatic absorption of LK-903 and LKA. Similar results have also been reported in the studies on the bioavailability of LK-903.²⁴⁾ It seems probable that lecithin works effectively on the formation of micelles which are necessary for the penetration of LK-903 into the mucosal cell wall²⁵⁾ and/or on the formation of chylomicrons which are important for lymphatic absorption of fat. On the other hand, the high dose of triolein decreased the absorption of LK-903 as shown by Aso *et al.*⁴⁾ These results can be explained by assuming that the high dose of triolein may have decreased the partition of LK-903 from the emulsion phase to the micellar phase.

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References

- 1) K. Takashima, K. Ohyama, T. Mori and S. Takeyama: Hypolipidemic effect of α -mono-*p*-myristyloxy- α -methylcinnamoyl glycerol (LK-903) in rats, *Biochem. Pharmacol.*, **27**, 2631–2635 (1978).
- 2) K. Takashima, T. Mori, K. Ohyama, S. Nitta and S. Takeyama: Hypocholesterolemic activities of LK-903 and clofibrate in miniature pigs, cynomolgus monkeys and beagle dogs, *Chem. Pharm. Bull.*, **26**, 2454–2457 (1978).
- 3) J. Sugihara, M. Fujii and M. Fujihara: Unpublished observations.
- 4) Y. Aso, M. Agata and K. Mishima: Chylomicra forming absorption of foreign compounds, Proceedings of the 12th Symposium on Drug Metabolism and Action, Kanazawa, 1980, pp. 86–89.
- 5) J. D. Kamp and H. G. Neumann: Absorption of carcinogens into the thoracic duct lymph of the rat: aminostilbene derivatives and 3-methylcholanthrene, *Xenobiotica*, **5**, 717–727 (1975).
- 6) Y. Aso, T. Arisaka, K. Mishima and H. Kitagawa: Thoracic duct transport of drugs and evaluation experimental procedures, Proceedings of the 2nd Symposium on Drug Metabolism and Action, Kyoto, 1970, pp. 27–30.
- 7) S. M. Sieber, V. H. Cohn and W. T. Wynn: The entry of foreign compounds into the thoracic duct lymph of the rat, *Xenobiotica*, **4**, 265–284 (1974).
- 8) R. C. Grimus and I. Schuster: The role of the lymphatic transport in the enteral absorption of naftifine by the rat, *Xenobiotica*, **14**, 287–294 (1984).
- 9) T. Meshi and Y. Sato: Preparation of tritium labeled compounds and their distribution in Animals (I). Tritium labeling of a new local anesthetic 2-methyl-2-*n*-propylaminopropion-*o*-toluide hydrochloride (LA-012), *Radioisotopes*, **16**, 251–259 (1967).
- 10) M. Fujihara and T. Suzuki: Unpublished observations.
- 11) P. Belfrage, J. Elovson and T. Olivecrona: Radioactivity in blood and liver partial glycerides, and liver phospholipids after intravenous administration to carbohydrate-fed rats of chyle containing double-labeled triglycerides, *Biochim. Biophys. Acta*, **106**, 45–55 (1965).
- 12) E. Baer and H. O. L. Fischer: Synthesis of a homologous series of optically active normal aliphatic α -monoglycerides (L-series), *J. Amer. Chem. Soc.*, **67**, 2031–2046 (1945).

- 13) Y. Aso: Cannulation into lymph duct of rat, "Absorption and Excretion of Drug," ed. by Y. Omori and T. Oda, Hirokawa Publishing Co., 1969, pp. 35—37.
- 14) V. D. Dole: A relation between non-esterified fatty acids in plasma and the metabolism of glucose, *J. Clin. Invest.*, **35**, 150—154 (1956).
- 15) L. I. Gidez and M. L. Karnovsky: The metabolism of glyceride-glycerol, *J. Biol. Chem.*, **223**, 293—306 (1956).
- 16) L. I. Gidez and M. L. Karnovsky: The metabolism of ^{14}C -glycerol in the intact rat, *J. Biol. Chem.*, **206**, 229 (1954).
- 17) B. Borgström: On the mechanism of pancreatic lipolysis of glycerides, *Biochim. Biophys. Acta*, **13**, 491—504 (1954).
- 18) F. H. Mattson, J. H. Benedict, J. B. Martin and L. W. Beck: Intermediates formed during the digestion of triglycerides, *J. Nutr.*, **48**, 335—344 (1952).
- 19) B. Borgström: Studies of intestinal digestion and absorption in the human, *J. Clin. Invest.*, **36**, 1521—1530 (1957).
- 20) P. Savary and P. Desnuelle: Sur quelques elements de specificite pendant L'hydrolyse enzymatique des triglycerides, *Biochim. Biophys. Acta*, **21**, 349—360 (1956).
- 21) J. M. Johnston and J. L. Brown: The intestinal utilization of doubly labeled α -monopalmitin, *Biochim. Biophys. Acta*, **59**, 500—501 (1962).
- 22) J. L. Brown and J. M. Johnston: The mechanism of intestinal utilization of monoglycerides, *Biochim. Biophys. Acta*, **84**, 264—274 (1964).
- 23) J. R. Senior and K. J. Isselbacher: Demonstration of an intestinal monoglyceride lipase: An enzyme with a possible role in the intracellular completion of fat digestion, *J. Clin. Invest.*, **42**, 187—195 (1963).
- 24) M. Mizobe, S. Matsuda, T. Yoneyama and K. Kohno: Evaluation of bioavailability of hypolipidemic compound LK-903, *Chem. Pharm. Bull.*, **31**, 2424—2431 (1983).
- 25) W. J. Simmonds, T. G. Redgrave and L. S. Willix: Absorption of oleic and palmitic acids from emulsions and micellar solutions, *J. Clin. Invest.*, **47**, 1015—1025 (1968).