Intestinal Absorption of Dolichol from Emulsions and Liposomes in Rats

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The intestinal absorption of dolichol from various dosage forms was investigated using the intestinal loop and everted sac methods in the rat. The *in situ* loop experiments showed that the absorption of dolichol from a triglyceride emulsion was dependent on the chain-length of the triglyceride; the absorption from a tri-*n*-butyrin emulsion in 1 h was 18.0% of the dose; and the absorption from an HCO-60 suspension was 4.3%. The liposomal preparation enhanced the absorption up to 39.1% of the dose. In *in vitro* experiments, 25.0% and 13.2% of dolichol were taken up by everted sacs of the jejunum and the ileum, respectively. On the other hand, phospholipids composing liposomes were not absorbed under these conditions. The above results suggest that the absorption mechanism from liposomal preparations may be as follows: dolichol is released from the liposomes into the aqueous phase adjacent to the surface of the intestine and is subsequently partitioned into the intestinal tissue.

Keywords dolichol; liposome; emulsion; intestinal absorption; rat

Introduction

There are several advantages to the oral administration route. However, some drugs cannot be administered *via* the oral route due to their poor absorbability from the gastrointestinal tract. Increased efforts have been made to improve the gastrointestinal absorption of such drugs. Ueno *et al.*[1] reported that oral administration of liposomally entrapped heparin to beagle dogs induced a prolongation of clotting time and increased the heparin activity in the blood. The effect of liposomally associated vitamin K_1 administered orally in rabbits with warfarin-induced hyperthrombinemia was investigated by Nagata *et al.*[2] These studies suggested that liposomes may be able to improve the gastrointestinal uptake of poorly absorbable drugs.

In the present study, we attempted to improve the absorption of a very hydrophobic model compound, dolichol, by means of a liposomal preparation. Dolichol (from the Greek "dolichos" meaning long) is an isoprenoid alcohol which is present in various mammalian tissues at low concentrations (0.04—3000 μg/g).[3] Dolichol constitutes an extremely long hydrocarbon molecule which is made up a single repeating unit (see Fig. 1). Dolichol extends to approximately 10 nm in length and normally occurs as a mixture with different chain-lengths. Dolichyl phosphate, a phosphorylated form of dolichol, plays an essential role as a carrier of oligosaccharide in the lipid intermediate pathway for glycoprotein synthesis in all eukaryotic cells. Recently, Shimamura *et al.*[4] reported that exogenous addition of dolichol or dolichyl phosphate significantly enhanced the colony formation of hematopoietic progenitors. The capabilities for therapeutic use of dolichol in anemia, however, have been restricted due to the low bioavailability after oral administration of the compounds. We report here the influence of various vehicles on the intestinal absorption of dolichol and discuss the absorption mechanisms.

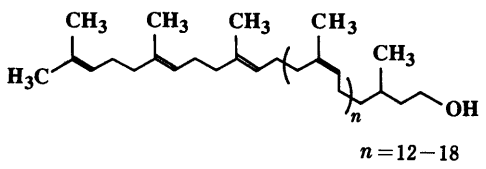

Fig. 1. Chemical Structure of Dolichol

Experimental

Materials Dolichol was supplied by the Central Research Laboratories of Kuraray Co., Ltd., Kurashiki, Japan.[5] The dolichol used in this study had a molecular-weight distribution similar to that in porcine liver; *n* of the main component was 15, and the average molecular weight was 1298.4. Soybean lecithin, egg yolk lecithin and distearoylphosphatidylcholine (DSPC) were supplied by Nihon Shoji Co., Ltd., Osaka, Japan. The dolichol and phospholipids were stored as stock solutions in chloroform at −20 °C under a nitrogen atmosphere. HCO-60 (hydrogenated castor oil, trioxyethylated) was supplied by Nikko Chemical Co., Ltd., Tokyo, Japan. Olive oil (Yamakei Co., Ltd., Osaka), tri-*n*-butyrin (Nakarai Chemicals, Ltd., Kyoto) and tri-*n*-caprylin (Wako Pure Chemical Industrials, Ltd., Osaka) were used as obtained. All other chemicals were of reagent grade and were used without further purification.

Preparation of Dolichol Aqueous Suspension Ten milligrams of dolichol was dried from the stock solution in a rotary evaporator and 25 ml of distilled water containing 0.5%(w/v) of HCO-60 was added. The dolichol was suspended by sonication using an ultrasonic homogenizer (type 5201, Ohtake Works, Tokyo, Japan) with a 100 W output power for 5 min on an ice-cold bath.

Preparation of Emulsions Containing Dolichol Five hundred milligrams of triglycerides was dissolved in the stock solution of dolichol (10 mg) and dried under reduced pressure. To the residue, 25 ml of 0.1% Tween-80 aqueous solution was added and the mixture was sonicated as described above.

Preparation of Liposomes Stock solutions containing 25 mg of phospholipids and 10 mg of dolichol were taken into a round-bottomed flask, dried in a rotary evaporator to yield a thin film and placed in a vacuum for 2 h to remove traces of chloroform. Liposomes were formed by adding 25 ml of distilled water to the dried lipid film followed by vigorous mixing for 10 min with a vortex mixer and left overnight at 5 °C. The resultant suspension was examined under an electron microscope by the freeze-fracture technique and was confirmed to have a multilamellar structure (not shown). The preparations were agitated again with the vortex mixer just before administration.

***In Situ* Absorption Study** An *in situ* loop method[6] was employed. Male Wistar rats weighing 180—250 g were anesthetized with pentobarbital sodium (45 mg/kg) given by i.p. injection and the rectal temperature was maintained at 37 °C. The small intestine was exposed by a midline incision and a cannula was inserted into the portion just before the ligament of Treitz. The luminal side of the intestine was gently rinsed with 20 ml of saline at 37 °C and a second cannula was inserted at the ileo-cecal junction. At the start of the experiment, 5 ml of a test solution containing dolichol at 37 °C was slowly injected through the proximal cannula and both ends of the cannulae were ligated with clamps. The bile duct was cannulated in all experiments. After 1 h, the residual fluid in the intestinal lumen was washed out with saline.The intestinal tissue was also removed and homognized with a blender. The dolichol remaining in the luminal fluid

and the tissue was determined by high-performance liquid chromatography (HPLC).

***In Vitro* Uptake by Intestinal Mucosa** *In vitro* everted sac experiments[7] were employed to investigate the absorption mechanism of dolichol. Ten centimeters of the jejunum or ileum was removed from the rat and everted. Both ends of the sac were ligated and 0.5 ml of saline was injected as a serosal solution. Liposomal preparation (10 ml) containing 0.8 mg of dolichol and 2 mg of phospholipids maintained at 37 °C was used as a mucosal solution. An aliquot of the mucosal solution was sampled periodically and the concentrations of dolichol and phospholipids were determined.

Analytical Methods The dolichol remaining in the luminal fluid and that taken up by the intestinal tissue were extracted with chloroform : methanol (2 : 1 by volume). The organic layer was dried under a vacuum at 40 °C and re-dissolved in *n*-hexane. An aliquot of the *n*-hexane solution was injected into the HPLC system. The HPLC analysis was carried out with an LC-5A pump (Shimadzu, Kyoto, Japan) and normal phase silica-gel column, Polygosil-60 (Chemco Scientific Co., Osaka, Japan), at ambient temperature. The detection was performed with an SPD-2A ultraviolet detector (Shimadzu) at 212 nm. The mobile phase consisted of *n*-hexane : 2-propanol (99.6 : 0.4 by volume) and the flow rate was 2.0 ml/min. Phospholipids were determined enzymatically using an Iatron PL-E(OM) kit (Iatron Co., Tokyo, Japan).

Results and Discussion

Absorption of Dolichol from Aqueous Suspension and Emulsions Since dolichol has been found in many mammalian tissues, the endogenous dolichol level in the intestine was determined. The concentration of dolichol in the rat small intestine before administration of exogenous dolichol was 8.87 ± 0.12 μg/g wet tissue (equivalent to 60 μg/rat). This value was only 3% of the administered dose and could, for practical purposes, be disregarded in these studies. *In situ* loop experiments were undertaken to examine the effects of vehicles on the absorption of dolichol. The levels of intact dolichol remaining in the luminal fluid and the intestinal tissue at 1 h after administration as the aqueous suspension and o/w emulsion are listed in Table I. The apparent absorption in 1 h is represented as the difference between the initial dose and the sum of the remainder in the lumen and the intestinal tissue. The absorption of dolichol from the HCO-60 suspension amounted to 4.3%, whereas the emulsion forms markedly increased the absorption of dolichol. The apparent absorption of dolichol from the emulsion of tri-*n*-caprylin was higher than that of olive oil. This difference may reflect the higher affinity of dolichol for olive oil and, in other words, a lower escaping tendency of dolichol from an oil with a long-chain. However, tri-*n*-butyrin which has a shorter fatty-acid chain did not exhibit any further significant enhancement of the absorption. The escaping tendency of dolichol from the tri-*n*-butyrin emulsion appeared to be higher than that from the tri-*n*-caprylin emulsion. However, the relatively rapid absorption rate of the short-chain triglyceride as reported by Noguchi *et al.*[8] could make the dolichol dispersion unstable. These opposite effects on the absorption of dolichol might result in the not-so-large enhancement.

TABLE I. Absorption of Dolichol from Various Dosage Forms by Rat Small Intestine

Vehicle	Dolichol remaining after 1 h			Absorption	*n*
	In lumen	In tissue	Overall		
Suspension HCO-60	82.2±7.4	13.4±4.2	95.7±2.5	4.3	5
Emulsion					
Olive oil	50.5±4.3	37.7±3.7	88.2±1.0[a)]	11.8	7
Tri-*n*-caprylin	47.5±6.8	36.1±8.2	83.6±1.4[a,b)]	16.4	3
Tri-*n*-butyrin	57.0±4.0	25.0±2.3	82.0±2.2[a−c)]	18.0	4
Liposome					
Soybean lecithin	27.0±2.0	33.9±1.7	60.9±2.1[a,d,e)]	39.1	11
Egg yolk lecithin	24.9±2.7	41.6±1.3	66.5±2.2[a,d,e)]	33.5	10
DSPC	42.7±2.0	43.7±4.2	86.4±2.1[a,d)]	13.6	3

Values are presented as percent of dose (mean±S.E.). Statistically significant differences between two studies were demonstrated. *a*) $p<0.01$ from suspension. *b*) $p<0.05$ from olive oil emulsion. *c*) Not significant different from tri-*n*-caprylin emulsion. *d*) $p<0.01$ from tri-*n*-butyrin emulsion. *e*) $p<0.01$ from DSPC liposomes.

Increased Uptake of Dolichol from Liposomal Preparations As can be seen from Table I, liposomal preparations composed of soybean lecithin and egg yolk lecithin enhanced the absorption of dolichol significantly more than the emulsions (up to 39.1% and 33.5% of dose, respectively), whereas dolichol in the liposomes prepared from DSPC was absorbed at only 13.6%. This difference may reflect the low fluidity of the DSPC liposome under the given experimental conditions. DSPC shows a main phase transition at 55 °C and DSPC liposomes would exist in the gel state at 37 °C, whereas soybean lecithin and egg yolk lecithin would exist in the fluid phase at the same temperature.[9] The release of dolichol from the gel-phase liposome would be slower than that from the fluid phase liposomes, resulting in a slower absorption.

Absorption Mechanism of Dolichol from Liposome Possible mechanisms of uptake of dolichol entrapped in liposomes by intestinal mucosa are shown schematically in Fig. 2. They can be summarized briefly as follows.

(a) Intact liposomes containing dolichol are taken up into epithelial cells by endocytosis.

(b) Liposomes interact or bind to surface of the brush border membrane during direct collison. Water is then excluded from the interface, and dolichol subsequently moves to the tissue directly.

(c) Dolichol is released from liposomes to the aqueous phase adjacent to the mucosal surface and partitions into the lipid phase of the brush border membrane.

Kimura *et al.*[7] demonstrated by using a water-soluble macromolecular marker compound that a small but significant amount of liposomes can be taken up intact with a relatively slow absorption rate by mucosal cells of the rat small intestine. Figure 3 illustrates the uptake of dolichol and soybean lecithin from liposomes by the jejunum and

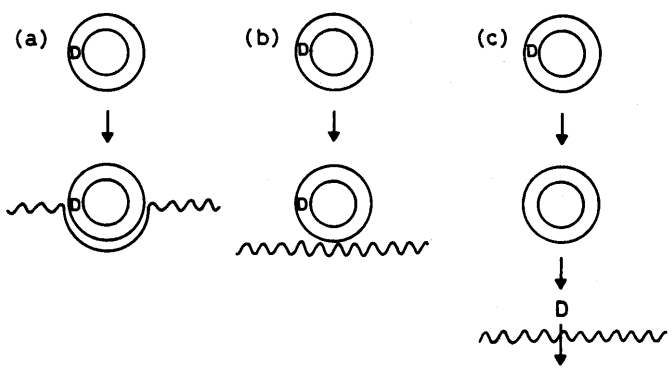

Fig. 2. Possible Mechanisms of Dolichol Uptake from a Liposomal Suspension by Mucosal Tissue

Double circles and "D" represent liposomes and dolichol molecules, respectively. See text for explanation of each mechanism.

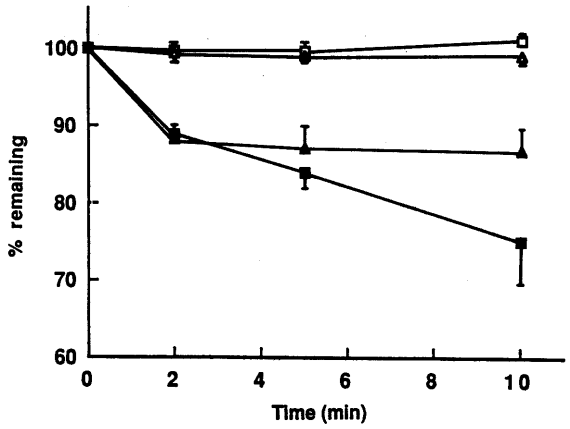

Fig. 3. Uptake of Dolichol and Soybean Lecithin from a Liposomal Suspension by Everted Jejunum and Ileum

□, lecithin incubated with jejunum; ■, dolichol incubated with jejunum; △, lecithin incubated with ileum; ▲, dolichol incubated with ileum. The results are expressed as the mean+or−S.E. of 4 experiments.

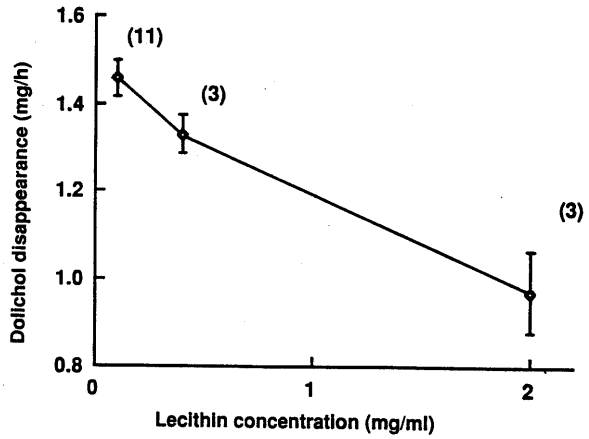

Fig. 4. Disappearance of Dolichol from the Luminal Solution as a Function of Soybean Lecithin Concentration

The results are expressed as the mean±S.E. with the number of experiments in parentheses.

the ileum *in vitro*. Dolichol was taken up rapidly in both portions, although the difference in mode of disappearance between the jejunum and ileum remains unexplained. On the other hand, phospholipids were practically not absorbed under these conditions. The above findings suggest that the uptake of intact liposomes containing dolichol might not play a principal role in the absorption process of dolichol from liposomes, so that, any contribution from mechanism (a) in Fig. 2 could be neglected.

Mechanisms (b) and (c) were assessed basically according to the analysis given by Westergaard and Dietschy[10] for the mucosal uptake of lipids from micelles of bile salts. The rate of dolichol uptake by the intestinal mucosa following the mechanism (b), J_b, may be expressed by the equation

$$J_b = P_{lm} \cdot C_l \cdot V_l \tag{1}$$

where P_{lm} is the permeability coefficient of dolichol from the liposome to the brush border membrane, C_l is the concentration of dolichol in the liposomes, and V_l is the volume of phospholipids in the liposomal suspension. By introducing the amount of dolichol in the liposomes, M_l, into Eq. 1, the following equation is obtained:

$$J_b = P_{lm} \cdot \left(\frac{M_l}{V_l}\right) \cdot V_l = P_{lm} \cdot M_l \tag{2}$$

Equation 2 means that the uptake rate is in proportion to the amount of dolichol in the liposomes.

On the other hand, the rate of dolichol uptake following mechanism (c), J_c, may be expressed by the equation

$$J_c = P_{wm} \cdot C_w \tag{3}$$

where P_{wm} is the permeability coefficient of dolichol from the aqueous phase to the brush border membrane, and C_w is the concentration of dolichol in the aqueous phase. Similarly, the following equation can be obtained from Eq. 3 by using the amount of dolichol in the aqueous phase, M_w, and the volume of the aqueous phase, V_w.

$$J_c = P_{wm} \cdot \left(\frac{M_w}{V_w}\right) \tag{4}$$

In the case of a dilute liposomal suspension, it may be permissible to consider that V_w is constant regardless of the phospholipid concentration. Thus, Eq. 4 means that the uptake rate is in proportion to the amount of dolichol in the aqueous phase.

The partition coefficient of dolichol between the aqueous phase and liposomes, K, can be given by

$$K = \frac{C_l}{C_w} \tag{5}$$

or

$$K = \left(\frac{M_l}{V_l}\right) \Big/ \left(\frac{M_w}{V_w}\right) \tag{6}$$

By using the total amount of dolichol, M_t, and the phospholipid concentration in %, L, M_l and M_w can be expressed by the following relationships:

$$M_l = \frac{K \cdot M_t}{\left(\frac{V_w}{V_l}\right) + K} = \frac{K \cdot M_t}{\left(\frac{100}{L}\right) + K} \tag{7}$$

$$M_w = \frac{M_t\left(\frac{V_w}{V_l}\right)}{\left(\frac{V_w}{V_l}\right) + K} = \frac{M_t}{1 + K\left(\frac{L}{100}\right)} \tag{8}$$

Equations 2 and 7 indicate that, if dolichol is absorbed by mechanism (b), an increasing phospholipid concentration in the system would result in an increase of dolichol uptake. On the other hand, Eqs. 4 and 8 indicate a contrary effect in the case of mechanism (c).

Figure 4 shows the effect of phospholipid concentration on the disappearance of dolichol from the intestinal lumen *in situ*. As can be seen, the uptake of dolichol was decreased with increasing phospholipid concentration. From these findings, the absorption mechanism of dolichol from the liposomal preparation is considered to be as follows: dolichol is released from the liposomes into the aqueous phase adjacent to the mucosal surface of the intestine and is subsequently partitioned into the lipid phase of the intestinal tissue.

The reason why liposomal preparation induced a greater enhancement effect on the absorption of dolichol than emulsions could be explained by a higher escaping tendency

of dolichol from liposomes as compared to that from emulsions. It has been reported that dolichol destabilizes the bilayer structure of liposomes.[11] This effect may result in a higher release rate of dolichol molecules from liposomes in the absorption process shown in Fig. 2 (c).

In the present study, experiments were carried out in a bile fistula condition in order to minimize changes in the liposomal structure. In normal animals, bile would solubilize the liposomes. However, since dolichol is a highly lipophilic compound, it would tend to be localized in the mixed micelles. The absorption mechanism might therefore not be so different from that described in this study. Studies on the fate of dolichol taken up by epithelial cells of the small intestine will be described in subsequent reports.

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