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Enhancement of Membrane Permeability to a Poorly Absorbed Drug by Medium-Chain Glycerides: Effect of Medium-Chain Glycerides on the Release of Phenol Red from Egg Phosphatidylcholine Liposomes

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The influence of medium-chain glyceride (MCG) on the membrane permeability was investigated. Liposomes with a basal composition of egg phosphatidylcholine : cholesterol = 4 : 1 were used to examine the effect of MCG and its components on the release of phenol red (PR) through the liposomal membrane by the dynamic dialysis method. With increase of MCG content in the membrane, the release of PR was enhanced. Among MCG components tested, dicaprylglyceride and tricaprylglyceride enhanced the release. When MCG emulsion was added to the control liposome suspension, PR was released more rapidly than from liposome without addition of MCG emulsion. To investigate the mechanism of the enhancement of membrane permeability, the effect of temperature on the release of PR was examined. The activation energies calculated from the slope of Arrhenius plots did not change significantly with the addition of MCG, so that it was unclear whether the change of membrane permeability could be correlated with the membrane fluidity.

Keywords——medium-chain glyceride; intestinal absorption; membrane permeability; liposome; phenol red

Introduction

The effectiveness of absorption promoters has been widely investigated.[1,2] In particular, lipoidal preparations have been tested to enhance the absorption of drugs from the gastrointestinal tract[3] and promising results were obtained with medium-chain fatty acids[4] and glycerides.[5] Although medium-chain fatty acids had been reported to have a chelating capacity[6] and to influence the protein function in brush border membrane,[7] the promoting mechanisms have not been elucidated in detail. The promoting effect of medium-chain glycerides (MCG) was ascribed to "the mild change in membrane,"[8] but again, the nature of the mechanism was obscure.

In the previous papers,[9,10] the effects of MCG emulsion on the intestinal absorption of bromthymol blue (BTB) and phenol red (PR) were reported. The absorption of PR was promoted more effectively, and *in vitro* everted sac studies suggested the enhancement of intestinal membrane permeability to PR.[10] In the present investigation, therefore, the influence of MCG on the membrane transport of drugs was examined with liposomes, which have often been used as a model membrane for studies on the transport of nutrients[11] or drugs.[12]

Materials and Methods

Materials——PR was purchased from Nakarai Chemicals. Polyoxyethylene derivatives of hydrogenated castor

oil (HCO-100), and MGK® (medium-chain glycerides; a mixture of mono-, di- and tricapryl glyceride and caprylic acid) were obtained from Nikko Chemicals. The averaged molecular weight of MGK® is 268.0. Components of MCG were fractionated stepwise as reported previously.[9,10] All other reagents were of analytical grade and were obtained from Nakarai Chemicals and Wako Pure Chemicals. Phosphatidylcholine (PC) was prepared from egg yolks and purified chromatographically on alumina and silica columns. It gave a single spot on thin-layer chromatography.

Preparation of Liposomes——Liposomes were prepared from a mixture of egg PC(80 μmol) and cholesterol (Ch; 20 μmol), with MCG or MCG components if necessary, according to the method of Bangham *et al.*[13] The lipid mixture dissolved in chloroform was pipetted into a 25 ml round-bottomed flask and rotary-evaporated under vacuum. The thin, dry lipid film was resuspended in 6 ml of PR solution (10.57 mM), using a Vortex mixer for 10 min. Under nitrogen, the suspension was sonicated (Ohtake Sonicator-150, Japan) for 2.5 min on ice. The liposomes were separated from free PR by gel filtration through a Sephadex G-50 column, and used immediately for the release experiment. Liposomes containing MCG or MCG components were eluted in almost the same filtrate fraction as the liposomes composed of egg PC and Ch. The sizes of liposomes ranged from 100 to 500 nm, based on observation with an electron microscope.

Measurement of the Release Rate from Liposomes——The release rate of PR from liposomes were determined according to the modified dynamic dialysis method of Klein *et al.*[14] Liposomal suspension (5 ml) was placed in a dialysis bag of Visking cellulose tubing (Visking Company 36/32 inch, D=2.7 cm) and the bag was transferred to a cuvette containing 60 ml of buffered saline (pH 6.5) at a controlled temperature. In some cases, 0.25 ml of MCG emulsion prepared as reported previously,[9] was added to the standard liposomal suspension (egg PC : Ch = 4 : 1) containing PR in the bag at the start of the experiment. At suitable time periods, 5 ml aliquots were taken from the incubation medium in the cuvette and replaced with an equivalent volume of buffered saline. As the permeability of the Visking bag is one order of magnitude higher than that of the liposomal membrane, the effect of the bag can be ignored in the overall release of PR. The release rate constant was calculated from the straight line[15] obtained for each set of data.

Analytical Methods——PR was alkalinized with 1 N NaOH and then determined spectrophotometrically at 560 nm. PR in liposomes was determined after the liposomes had been solubilized in 10% Triton X-100.

Statistical Analyses——Statistical analyses were performed by using the Student's *t*-test and the iterative non-linear least squares method with "MULTI."[16]

Results and Discussion

To study the effect of MCG on the membrane permeability, the release of PR from liposomes containing MCG as a membrane constituent was investigated (Fig. 1). With increase of the MCG molar ratio to egg PC, the release rate of PR from liposomes gradually

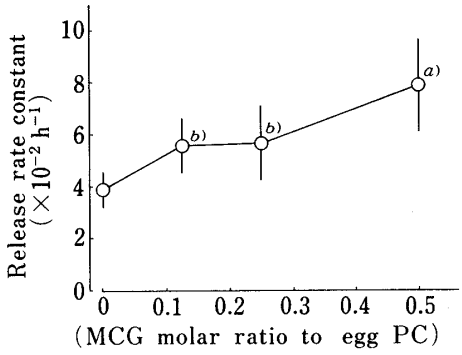

Fig. 1. Effect of MCG on the Release of PR from Liposomes

Release rate constants were calculated from the slope of the semilogarithmic plots of the dynamic dialysis data for 90 min. Data are the means ± S.D. of more than three experiments. Statistically significant differences between experimental groups and the control (MCG molar ratio is zero) are indicated as follows: *a*) $p<0.01$, *b*) $p<0.1$.

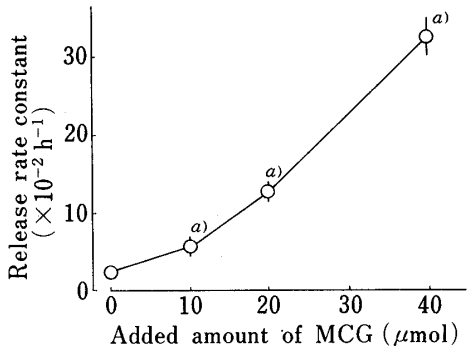

Fig. 2. Effect of Addition of MCG Emulsion on the Release of PR from the Liposomes (Egg PC : Ch = 4 : 1)

MCG emulsion (0.25 ml) containing various amounts of MCG was added to the control liposome (egg PC : Ch = 4 : 1) and release of PR from them was determined by the dynamic dialysis method. Release rate constants are the means ± S.D. of more than three experiments. Statistically significant differences between experimental groups and the control are indicated as follows: *a*) $p<0.001$.

increased to about twice the control at the molar ratio of 0.5, suggesting that MCG present in the membrane would influence the membrane and enhance the permeability. The uptake of PR into liposomes was also enhanced by the addition of MCG (data not shown).

On the other hand, MCG emulsion added to the bulk phase also promoted the release of PR in the MCG dose range of 10 to 40 μmol (Fig. 2), suggesting that MCG could influence on the permeability of the liposomal membrane from the outside of the liposome. The action of additional MCG can be ascribed to two factors: i) an interfacial effect of MCG emulsion in the bulk phase on the liposomal surface, and ii) an effect provoked by MCG present in the membranous lipids after transfer into the liposome. The greater effect of addition of MCG emulsion than preceding liposomal incorporation of MCG may suggest a larger contribution of the interfacial action.

The release of PR from liposomes was changed when an MCG component was incorporated into liposomes as a membrane constituent (Table I). Dicaprylglyceride and tricaprylglyceride increased the release of PR from liposomes more than did MCG itself. On the other hand, monocaprylglyceride and caprylic acid did not enhance the PR release from liposomes. These tendencies were observed in PR uptake experiments (data not shown). It was reported that the absorption of cefmetazole was most effectively promoted by monoglyceride when administered in the form of an oily solution in an *in situ* rectal absorption study.[17] However, monocaprylate did not increase the release of PR in the present investigation. Although it was suggested that there was an optimal content of monocaprylglyceride in MCG mixture for the promoting action,[10,17] the important factor for the promoting effect might be the ratio of other components, dicapryl- or tricaprylglyceride, to monocaprylglyceride.

The change in the membrane permeability is thought to coincide with that in the membrane fluidity, and an increase of the membrane fluidity is often accompanied with a decrease of the activation energy.[18] However, few studies on the effect of MCG have been reported. To investigate the mechanism of the change in the permeability of the liposomal membrane, the temperature dependency of the PR release constant was examined in various liposome preparations and the activation energies for PR permeation through the lipo-

Table I. Effect of MCG Components on the Release of Phenol Red from Liposome

Release rate constant	MCG components					
	Cont.	MCG	MG	DG	TG	CA
k ($\times 10^{-2}$ h^{-1})	3.94±0.73	5.62±1.57	2.93±0.20	15.34±2.00	6.60±0.46	2.93±0.57
p	—	<0.1	<0.01	<0.001	<0.01	<0.5

Release rate constants were obtained from dynamic dialysis studies. The composition of liposome is egg PC : Ch : X = 4 : 1 : 1, in which X is MCG or one of the MCG components, MG (monoglyceride), DG (diglyceride), TG (triglyceride), CA (caprylate). Data are mean values ± S.D. of more than three experiments.

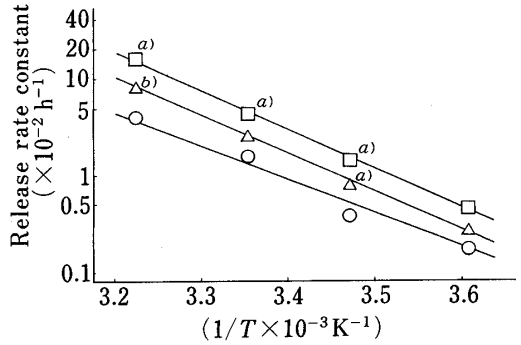

Fig. 3. Arrhenius Plot for the Release Rate Constants of PR from Liposomes

Each point is the mean value ± S.D. of at least four experiments. Regression lines were obtained from a least-squares fit of the points. Statistically significant differences between experimental groups and the control are indicated as follows: *a*) $p<0.001$, *b*) $p<0.01$. △, egg PC : Ch : MCG = 4 : 1 : 2; □, egg PC : Ch : DG = 4 : 1 : 1; ○, egg PC : Ch = 4 : 1.

somal membranes were calculated. Studies were performed on liposomes containing MCG (MCG-liposomes; egg PC:Ch:MCG=4:1:2) or dicaprylglyceride (DG-liposomes; egg PC:Ch:DG=4:1:1), which most effectively enhanced the release of PR (Fig. 3). At all temperatures, the release rates from both liposomes were greater than those from the control liposomes. Moreover, DG-liposomes showed greater release rates than MCG-liposomes. The activation energies (ΔE) calculated for the release of PR were 17.71 kcal/mol for MCG-liposomes, 18.31 kcal/mol for DG-liposomes, and 16.15 kcal/mol for the control liposomes. However, the differences are not statistically significant. In the present case, the release of PR from liposomes was promoted by MCG at each temperature, but there was no decrease of the activation energy. The activation energy tended to be greater in the case of MCG- or DG-liposomes than in control liposomes, although the differences were not statistically significant. Since the melting point of MCG, about 27 °C, is higher than that of egg PC, it is expected that the transition temperature of MCG-liposomal membrane would increase and the fluidity of the membrane would decrease compared to those of the control liposomes. It is difficult to explain the enhancement of PR release from the point of view of activation energies at present.

The changes of membrane fluidity in these liposomal preparations must be clarified by the use of other techniques.[19] Moreover, other mechanisms than the increase of membrane fluidity might be at least partly responsible for the increment in the membrane permeability to PR.

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