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# Effect of Cerivastatin on Permeability of Lipid-raft-mimetic Membranes

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***Author's contribution***

*The sole author designed, analysed, interpreted and prepared the manuscript.*

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## ABSTRACT

The effect of cerivastatin (CER) on the permeability of lipid-raft-mimetic membrane was investigated. CER is a drug candidate to treat dyslipidemia and cardiovascular diseases. Sphingomyelin (SM) was used to form the outer layer of liposomes of which inner layer was prepared with dioleoylphosphatidic-acid. The permeability of the SM layer was estimated by measuring the release of the pyranine encapsulated in the liposome. The change of the permeability was analyzed with the presence of CER dispersion in PBS buffer, especially its hydrophobic group. The results of this study seem to be useful in deriving the drug candidate for cardiovascular disease.

*Keywords: Cerivastatin; permeability; lipid raft; sphingomyelin; liposome.*

## 1. INTRODUCTION

"The model of biological membranes, such as liposomes, provides a convenient method to investigate the effects of drugs, toxins, and other substances ingested into the body" [1]. In the middle of the biological membrane composed of lipids, proteins, and sugar residues, the ordered microdomains called lipid rafts are unique and critical for its function [2]. It is known that the main component of the lipid raft is sphingomyelin (SM) [3]. Introduction.

"The lipid raft has been related to the function of the transmembrane protein called 3-hydroxy-3-methyloglutaryl-coenzyme A reductase of which

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regulation is necessary for the prevention and treatment of dyslipidemia and cardiovascular diseases and can be performed by means of statins that include hydrophobic group capable of binding and penetrating the lipid membranes" [4-7]. "Among statins, cerivastatin (CER) is a half-hydrophilic and half-hydrophobic drug with the octanol–water partition coefficient log P equal to 3.40–4.15" [8]. "CER is characterized by two $pK_a$ values: the lower one corresponding to the carboxylic acid form ($pK_a$ = 4.38) and the higher value to the pyridine residue ($pK_a$ = 5.29)" [9]. CER has been withdrawn from the pharmaceutical market in 2001 because the deaths were reported due to the side effect of rhabdomyolysis [10]. "This may be caused by the ability of this drug to deeply penetrate the membranes, where it ends up in the $CH_3$-terminal ends of the hydrophobic chains" [11].

Membrane permeability is the passive diffusion rate across the membrane. The common method to measure the permeability is to use liposomes, spherical lipid-bilayers, that encapsulate an indicator inside their aqueous region [12]. The permeability is able to suggest the characteristics of the membranes and the effect of their neighboring-agents [13]. Therefore, in this study, it is aimed to investigate the effect of CER on the permeability of the SM membranes.

## 2. MATERIALS AND METHODS

### 2.1 Liposome Preparation

Dioleoylphosphatidic acid (DOPA), dioleoylphosphatidylcholine (DOPC), sphingomyelin (SM), cerivastatin (CER), and pyranine were purchased from Sigma Aldrich (St. Louis, MO). These reagents were used without further purification. The DOPA was dissolved in 10 mL of *tert*-butyl methyl ether at 10 mg/mL, followed by adding 100 μL deionized-water of 25 mM pyranine, 10 mM Tris-HCl at pH 7.0. Therefore, the micelles with DOPA were prepared by extrusion through the 50 nm pores of 78 mm diameter PTFE membranes above the transition temperature of DOPA. Several drops (less than 10 μL) of the micelle solution and *tert*-butyl methyl ether solution of 10 mg/mL DOPC or SM were continuously added through a 22-gauge needle inserted into the 10 mL aqueous solutions of 10 μM CER and 10 mM Tris-HCl at pH 8.0, respectively. The final lipid concentration of the aqueous solution was 1 mg/mL. During the addition, the solution was magnetically stirred under the nitrogen stream. The liposome solution was acquired from the supernatant of the solution that underwent through the centrifugation (3700 × g). These procedures are well known as a way to prepare vesicles [14].

### 2.2 Permeability Measurements

The fluorescence intensity was measured in real time with a Wallac Victor3 multiwell fluorimeter (Perkin-Elmer, Waltham, MA), because the intensity of the pyranine increased with the higher value of its environmental pH. ANTS has different fluorescence intensities. Three kind of liposome were considered for the measurements according to the components and the environment – DOPA/DOPC, DOPA/SM, and DOPA/SM exposed to CER. As described in previous section, DOPA was used to form the inner layer of the liposome.

## 3. RESULTS AND DISCUSSION

### 3.1 Liposome Characterization

To confirm the liposome formation, the diameters of the micelles and the liposomes were measured with a light scattering (ELS-8000; Otsuka Electronics, Osaka, Japan). For the measurement of the micelle, the viscosity and the refractive index of the *tert*-butyl methyl ether are 0.23 cP and 1.3686, respectively. The diameter of the micelles was 75 ± 10 nm, and the diameter of the liposomes became 80 ± 10 nm as expected from the lipid layer formed on the micelle surface. The encapsulation of the pyranine was confirmed with a fluorometer (Fig. 1). The fluorescence intensity was changed with Tween 20 treatment. Without this treatment, the intensity did not vary after the addition of distilled water drops at pH 3. Thus, encapsulation was successfully achieved.

### 3.2 Permeability Measurements

More pyranine release was observed from the liposomes prepared with the DOPA and DOPC than those with the DOPA and SM due to the presence of the unsaturation. Since the inner layer was DOPA only for both liposomes, the difference in the release was caused by the characteristics of the outer layer only. DOPC has double bond in its structure as DOPA, while SM does not. The more release indicated that the layers of the liposomes were more permeable. Therefore, the less permeability of the DOPA:SM liposomes was from SM only. After the liposomes with the DOPA and SM were exposed to the

solution containing CER, the intensity decreased compared to before the exposure (Fig. 2). However, the intensity little changed for the liposomes with the DOPA and DOPC. From this observation, it was found that the CER appeared to increase the permeability of the SM layer only.

The increase in the permeability seems from the interaction between the layer and CER. Since the hydrophobicity of CER was the motif to penetrate the SM layer, it is believed that the permeability of SM layer increased. The unsaturated lipids such as DOPA and DOPC have the high permeability inherently, and CER has little effect their permeability although it may also interact with them. The penetration has been believed to disturb the arrange the lipid layer. Since the unsaturated-lipid layer was less dense, the disturbance of each lipid was relatively less for the unsaturated-lipid layer. This interpretation has been identical with the analysis of the research performed previously [6]. Furthermore, the interpretation seems consistent with CER's mediation to translocate membrane proteins [7].

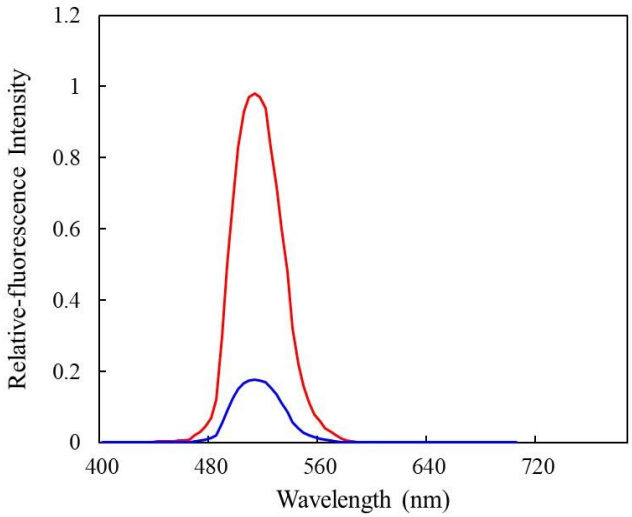


**Fig. 1. Fluorescence intensity change after the addition of pH 3 distilled water drops. Red: with detergent and Blue: without detergent**

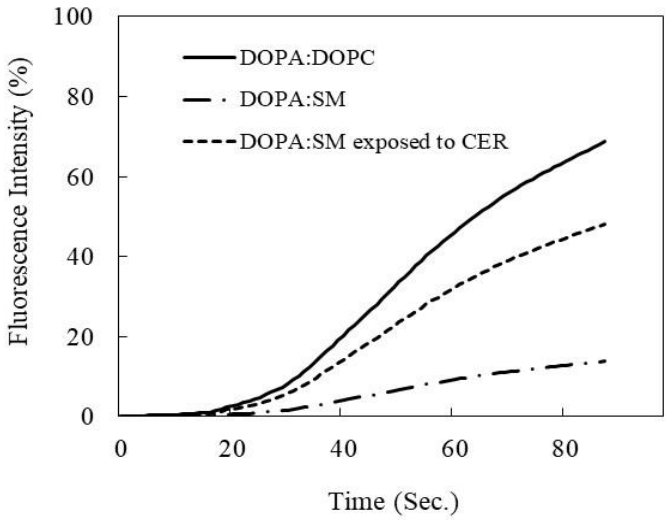


**Fig.2. Fluorescence intensity change caused by permeability of liposomes**

## 4. CONCLUSION

In this study, the effect of cerivastatin (CER) on the permeability of lipid-raft-mimetic membrane was investigated. Sphingomyelin (SM) was used to form the outer layer of liposomes of which inner layer was prepared with dioleoylphosphatidic-acid. The permeability of the SM layer was estimated by measuring the release of the pyranine encapsulated in the liposome. The change of the permeability was analyzed with the presence of CER dispersion in PBS buffer. The results of this study seem to be useful in deriving the drug candidate for cardiovascular disease.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

## REFERENCES

1. Park Y, Park JW. Specific detection of pe-included vesicles using cyclic voltammetry. Appl. Sci. 2021;11:3660.
Available:https://doi.org/10.3390/app11083660.
2. Wang HY, Bharti D, Levental I. Membrane heterogeneity beyond the plasma membrane. Front. Cell Dev. Biol. 2020;8:580814.
Available:https://doi.org/10.3389/fcell.2020.580814.
3. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science 2010;327:46-50.
Available:https://doi.org/10.1126/science.1174621.
4. Corsini A. The safety of hmg-coa reductase inhibitors in special populations at high cardiovascular risk. Cardivasc. Drugs Ther. 2003;17:265–285.
Available:https://doi.org/10.1023/a:1026132412074.
5. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292:1160-1164.
Available:https://doi.org/10.1126/science.1059344.
6. Zaborowska M, Broniatowski M, Fontaine P, Bilewicz R, Matyszewsk D. Statin action targets lipid rafts of cell membranes: GIXD/PM-IRRAS investigation of langmuir monolayers. J. Phys. Chem. B 2023;127:7135-7147.
Available:https://doi.org/10.1021/acs.jpcb.3c02574
7. Sinnett-Smith J, Torres-Marquez ME, Chang JK, Shimizu Y, Hao F, Martin MG, Rozengurt E. Statins inhibit protein kinase D (PKD) activation in intestinal cells and prevent PKD1-induced growth of murine enteroids. Am. J. Physiol. Cell Physiol. 2023;324:C807-C820.
Available:https://doi.org/10.1152/ajpcell.00286.2022.
8. Kim SH, Park Y, Matalon S, Franses EI. Effect of buffer composition and preparation protocol on the dispersion stability and interfacial behavior of aqueous DPPC dispersions. Colloids Surf. B: Biointerfaces 2008;67:253-260.
Available:https://doi.org/10.1016/j.colsurfb.2008.09.003.
9. Adams EM, Casper CB, Allen HC. Cation enrichment on dipalmitoylphosphatidylcholine (DPPC) monolayers at air-water interface. J. Colloid Interface Sci. 2016;478:353-364.
Available:https://doi.org/10.1016/j.jcis.2016.06.016.
10. Galiullina LF, Musabirova GS, Latfullin IA, Aganov AV, Klochkov VV. Spatial structure of atorvastatin and its complex with model membrane in solution studied by Nmr and theoretical calculations. J. Mol. Struct. 2018;1167:69-77.
Available:https://doi.org/10.1016/j.molstruc.2018.04.012.
11. Galiullina LF, Aganova OV, Latfullin IA, Musabirova GS, Aganov AV, Klochkov VV. Interaction of different statins with model membranes by NMR data. Biochim. Biophys. Acta – Biomembr. 2017;1859:295-300.
Available:https://doi.org/10.1016/j.bbamem.2016.12.006.
12. Park JW. Phase asymmetry effect on vesicle fusion induced by phospholipase D. Korean Chem. Eng. Res. 2015;53:672-676.

Available:http://dx.doi.org/10.9713/kcer.2015.53.6.672.

13. Park JW. Effect of trehalose on biological membranes with respect to phase of the membranes. KSBB J. 2017;32:103-107. Available:http://dx.doi.org/10.7841/ksbbj.2017.32.2.103.
14. New RRC. Liposomes: A practical approach. Academic Press, New York, USA. 1990;20-41.