Enhanced Enteral Bioavailability of Vancomycin Using Water-in-Oil-in-Water Multiple Emulsion Incorporating Highly Purified Unsaturated Fatty Acid

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Abstract: The aim of this study was to evaluate the potential of an emulsion incorporating unsaturated fatty acids to improve the mucosal absorption of poorly absorbed drugs from rat intestinal loops in situ, using a water-in-oil-in-water (W/O/W) multiple emulsion. Vancomycin hydrochloride (VCM) was used as a model drug with low oral bioavailability. The entrapment efficiency of VCM in the emulsion was ~60% and remained constant over storage for 1 month at 4°C. The emulsion incorporating C18 unsaturated fatty acids or docosahexaenoic acid (DHA) markedly enhanced VCM absorption after colonic and rectal dosing. The effectiveness of DHA on VCM colonic absorption improvement was the same as that of oleic acid, and less than that of linoleic and linolenic acids. For rectal dosing, bioavailability was similar among various emulsions, in the range 40–50%. The effect of the emulsion incorporating oleic acid or DHA on improving VCM enteral bioavailability was not increased proportional to the incorporated amount. The electrical resistance of membranes was not changed by the incorporation of various fatty acids in emulsions. Our results indicated that W/O/W emulsions incorporating C18 unsaturated fatty acid or DHA were useful carriers for improving the absorption of poorly absorbable drugs via the intestinal tract without gross changes to tight junction function. © 2000 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 89:1243-1252, 2000.

Keywords: vancomycin; docosahexaenoic acid; unsaturated fatty acid; emulsion; intestinal absorption

Introduction

The effective oral delivery of a drug requires that the drug have sufficient solubility, sufficient stability in the gastrointestinal (GI) tract, and the ability to pass through the intestinal wall. Small amphipathic drugs move efficiently through the transcellular route by partitioning into and out of lipid bilayers. Small hydrophilic drugs are restricted to the paracellular route, and are therefore often poorly absorbed when administered orally. There has been considerable interest in the possibility of using absorption enhancers, such as surfactants, bile acids, fatty acids, and chelating agents, to promote absorption of polar molecules across the intestinal wall.1 Many therapeutic compounds, such as antibiotics and peptide and protein drugs, require the use of some kind of absorption enhancer to obtain reasonable plasma concentrations. Nevertheless, the safety profile is of primary importance for clinical use of absorption enhancers. We previously reported...
that water-in-oil-in-water (W/O/W) multiple emulsion incorporating polyunsaturated fatty acids\(^2\) and long-chain polyunsaturated fatty acids\(^3\,4\) strongly enhanced intestinal absorption of the protein drug insulin. Particularly, polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), were more effective than unsaturated fatty acids with a C18 alkyl chain for improving insulin mucosal absorption,\(^3\) and insulin dose-related pharmacological effects could be obtained in in vivo absorption studies with the emulsion incorporating DHA.\(^4\) Histological\(^2\,3\) and biochemical\(^4\) studies demonstrated that the emulsion did not induce gross damage to the intestinal mucosa, therefore, the W/O/W emulsions incorporating unsaturated fatty acid were considered to be safe and useful carriers for enhancing insulin enteral absorption. However, the effect of the emulsion on the mucosal absorption of other poorly absorbed drugs has not been elucidated.

In the present study, we evaluated the potential application of the emulsion to a water-soluble drug with low oral bioavailability, and selected vancomycin hydrochloride (VCM) as a model drug. VCM, a glycopeptide antibiotic for treatment of infections with methicillin-resistant staphylococci, is water-soluble and poorly absorbed from GI tract.\(^5\) The VCM emulsions incorporating unsaturated fatty acids were prepared and the VCM entrapment efficiency was determined. We evaluated the improvement in colonic and rectal VCM absorption. The enteral bioavailability of VCM was calculated by defining the relationship between dose and area under the curve of concentration versus time (AUO) following intravenous (iv) administration. The pharmacokinetic parameters were calculated and compared among a range of fatty acids. In addition, we evaluated the influence of the amount of unsaturated fatty acid in the oily phase on absorption enhancement. To develop effective and safe promoters for drug absorption from the GI tract, it is necessary to estimate the effects quantitatively and to characterize the mechanism of action. The lipoidal absorption enhancers may enter the lipid bilayer structure in the intestinal brush-border membrane and disrupt the configuration of the lipid region.\(^6\) Consequently, the intestinal membrane might become more permeable to drug entrapped in the inner aqueous phase of the W/O/W emulsion. On the other hand, recent study shows a regulatory effect of polyunsaturated fatty acids on tight junction function.\(^7\) However, the effects of the emulsion incorporating unsaturated fatty acid on tissue membrane resistance \((R_m)\) have not yet been evaluated. Therefore, changes in \(R_m\) of rat intestinal mucosa during application of the emulsions were evaluated in Ussing chamber experiments.

MATERIALS AND METHODS

Materials

Vancomycin hydrochloride, gelatin, triolein, DL-\(\alpha\)-tocopherol, sorbitan monoleate (Span 80), and polyoxyethylene sorbitan monoleate (Twee 80) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Oleic acid (purity: 99.0%) and sodium caprate were purchased from Tokyo Kasei Kogyo Company, Ltd. (Tokyo, Japan). Egg yolk phospholipids (phosphatidylcholine and phosphatidylethanolamine) were purchased from Nippon Oil & Fats Company, Ltd. (Tokyo, Japan). Linoleic acid (purity: >99.0%), linolenic acid (purity: 98.0%), and ethylenediaminetetraacetic acid were obtained from Sigma Chemical Company, Ltd. (St. Louis, MO). Docosahexaenoic acids (purity: 90.0 and 97.0%) and eicosapentaenoic acid (purity: 97.0%) were provided by Nippon Suisan Kaisya, Ltd. (Tokyo, Japan). All other chemicals were of analytical grade and commercially available. Spectra/Por\(^\text{R}\) CE (cellulose ester dialysis membrane MWCO 50,000) were purchased from SPECTRUM (Rancho Dominguez, CA).

Preparation of W/O/W Emulsion

The W/O/W emulsions were prepared by a two-step emulsification procedure using a homogenizer (Ace Homogenizer, Nihonseiki Kaisha Ltd., Tokyo, Japan) according to the method reported previously.\(^2\) VCM dissolved in 5% gelatin solution was used for the inner aqueous phase. The oily phase was composed of 0.0625% DL-\(\alpha\)-tocopherol, 5% egg yolk phospholipids (phosphatidylcholine:phosphatidylethanolamine = 7:3), 10% free unsaturated fatty acid, 20% Span 80, and 64.937% triolein. Each unsaturated fatty acid was added to the oily phase. In the study of effects of fatty acid amount in the oily phase on absorption enhancement, DHA of 90% purity was used for preparing the emulsions. The purified water containing 3% Tween 80 was used for the outer aqueous phase. The weight ratio of each phase was as follows: inner aqueous phase:oily phase:outer aqueous phase = 1:4:15.
Determination of Entrapment Efficiency of VCM in the W/O/W Emulsion

The W/O/W emulsion (3 g) incorporating 2% oleic acid was put in a dialysis membrane (Spectra/Por®) and dialyzed to normal saline at 4 or 37 °C. The dialyzed solution was sampled immediately after preparation, 1, 4, 7, and 28 days, and the concentration of VCM was measured. The entrapment efficiency of VCM in the W/O/W emulsion (%) was calculated as (dialyzed amount of VCM)/(theoretical amount of VCM) x 100.

In Situ Absorption Experiments

Male Wistar rats weighing 170–210 g were fasted for 48 h prior to the experiments and then anesthetized by intraperitoneal (ip) injection of 50 mg/kg sodium pentobarbital. Rats were restrained in the supine position on a board that was kept at a surface temperature of 37 °C. A small midline incision was made in the abdomen. A 5-cm loop of colon or the rectum was identified and ligated at both ends. The colon loop was made at the ascending colon. The emulsion (0.5 g) was administered directly into the loop. The dose of VCM was fixed at 5 mg/kg body weight. The VCM emulsion without unsaturated fatty acid was used as control. Approximately 5 min before administration, a 0.2-mL aliquot blood sample was taken from the jugular vein. Subsequent blood samples were taken at 5, 15, 30, 60, 120, 180, 240, and 360 min after dosing. Plasma was separated by centrifugation at 13,000 rpm for 3 min and kept frozen until analysis. The plasma VCM concentration was determined by a fluorescence polarization immunoassay (TDX System, DAINABOT Company, Ltd., Tokyo, Japan). The plasma peak concentration (Cmax) and the time to reach plasma peak concentration (Tmax) were determined from the plasma VCM concentration–time curves. The area under the VCM concentration–time curves for 0–6 h (AUC) were determined with the trapezoidal rule. Mean residence time (MRT) was calculated by dividing AUMC by AUC, where AUMC is the area under the first moment curve for insulin from zero to the 6-h point.

The extent of bioavailability following colonic and rectal administration was calculated from the relationship between dose and AUC following the iv experiment. In the experiment, VCM solution was administered iv via the jugular vein. The iv VCM doses were 2.5, 5, and 10 mg/kg body weight. Blood samples were collected from the jugular vein on the opposite side to the injection before and at 3, 5, 15, 30, 60, 120, and 180 min after dosing.

The experimental procedures just described were performed according to the rules set by the Committee on Ethics in the Care and Use of Laboratory Animals in Hoshi University.

Measurement of Membrane Electrical Resistance

Male Wistar rats weighing 170–210 g and fasted for 48 h prior to the experiments were anesthetized by ip injection of 50 mg/kg sodium pentobarbital. The colon or the rectum was rapidly removed from rats, and opened along the mesenteric border to give a flat sheet. After washing the intestinal contents with ice-cold Ringer solution, which was continuously gassed with a O2:CO2 (95:5) gas mixture, the muscle layer of the membrane was stripped using a microscope slide glass and forceps. The composition of the standard Ringer solution was: 125.0 mM NaCl, 5.0 mM KCl, 1.4 mM CaCl2, 1.2 mM NaH2PO4, 10.0 mM NaHCO3, and 11.0 mM D-glucose (pH 7.4). Care was taken to avoid the Peyers patches. During preparation, tissues were submerged in continuously gassed, ice-cold Ringer solution. The mucosal sheet that consisted of the epithelial layer and underlying connective tissue was immediately mounted in Ussing-type chambers on a segment holder, with a surface area of 0.67 cm² (Nihon Kohden Tokyo Company Ltd., Tokyo, Japan). Both sides of the membrane in the chamber were filled with 10 mL of Ringer solution and stirred by bubbling with the gas mixture. After a 10-min incubation, 1 mL of Ringer solution in the mucosal side was replaced by 1 mL of various test emulsions. These experiments were performed under temperature-controlled conditions at 37 °C. For measurements of the short-circuit current, the transmucosal electrical potential differences were maintained at 0 mV by an automatic voltage clamp system. The tissue electrical resistance was calculated by Ohm’s law, taking into account the calculated resistance of the bathing solution. The effect of 50 mM ethylenediaminetetraacetic acid (EDTA) and 50 mM sodium caprate on Rm were estimated as active controls.

Statistical Analysis

For group comparisons, the one-way layout ANOVA with duplication was applied. Significant differences in the mean values were evaluated by
Table 1. Changes in Percent Vancomycin Entrapment Efficiency

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Days After Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>4 °C</td>
<td>65.3 ± 3.7</td>
</tr>
<tr>
<td>37 °C</td>
<td>54.9 ± 7.1</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± SE of 4 determinations.

RESULTS AND DISCUSSION

VCM Entrapment Efficiency

Table 1 shows the percent VCM entrapped in the inner aqueous phase of the emulsion. The entrapment efficiency was ~60% when the emulsion was stored at 4 °C during the experimental period. The values remained constant over 1 month of storage. Improvement of the stability of the W/O/W emulsions by gelatin has been reported.10,11 Aggregation of the aqueous droplets in the oily phase was prevented by the addition of gelatin to the inner phase.11 However, VCM entrapped efficiency of the emulsion stored at 37 °C gradually decreased over the experimental period. Under these conditions, the phase separation started 7 days after preparation. Gelatin in the inner phase became sol at 37 °C; therefore, rupture of the inner aqueous phase might have been accelerated under these conditions. These results are consistent with our previous findings using the emulsion incorporating glucose, a marker substance, by measuring the turbidity.12 In addition, our recent study indicated that pharmacological availability of W/O/W multiple insulin emulsion stored at 4 °C did not change during multiple administration study for 10 days.15 The experimental evidence and the results obtained in this study suggest that the emulsion stored at 4 °C is stable and does not leak the drug entrapped in the inner aqueous phase, at least over a period of 1 month.

Effects of W/O/W Emulsion Incorporating Unsaturated Fatty Acid with a C18 Alkyl Chain on Intestinal VCM Absorption

The VCM plasma concentration following administration of the emulsion incorporating unsaturated fatty acids with an 18 carbon alkyl chain into the colon loop are shown in Figure 1. VCM could not be absorbed from the emulsion without unsaturated fatty acid. On the other hand, VCM plasma concentrations markedly rose following administration of the emulsion incorporating un-

![Figure 1. Vancomycin plasma concentration versus time following intracolonic administration of vancomycin emulsion incorporating C18 unsaturated fatty acid. Key: (●) emulsion incorporating fatty acid; (○) control. Each value represents the mean ± SE (n = 3–7).](image-url)
Saturated fatty acid. Similarly, VCM plasma concentrations rose by rectal administration of the emulsion incorporating C18 unsaturated fatty acid (Figure 2). These findings suggest that the emulsion incorporating C18 unsaturated fatty acids is effective in improving enteral absorption not only for insulin, but also for VCM.

Effects of W/O/W Emulsion Incorporating DHA on Intestinal VCM Absorption

DHA is present in ω3-polyunsaturated fatty acid of fish oil, and has been found to exhibit various biological actions. In addition to many pharmacological effects, an influence of DHA on biological membranes has been proposed. Recently, we found a predominant promoting effect of highly purified DHA on insulin absorption from the colon and rectum. Similar to the insulin absorption enhancement effect, the colonic and rectal absorption of VCM was markedly enhanced by DHA (Figure 3). These findings suggest that the emulsion incorporating DHA would also be effective for improving absorption of another drug with low enteral absorption.

Figure 2. Vancomycin plasma concentration versus time following intrarectal administration of vancomycin emulsion incorporating C18 unsaturated fatty acid. Key: (●) emulsion incorporating fatty acid; (○) control. Each value represents mean ± SE (n = 4–7).

Figure 3. Vancomycin plasma concentration versus time following intracolonic and intrarectal administration of vancomycin emulsion incorporating DHA. Key: (●) emulsion incorporating DHA; (○) control. Each value represents the mean ± SE (n = 6).
Table 2. Comparison of Pharmacokinetic Parameters of Vancomycin Following Enteral Administration of Vancomycin Emulsion

<table>
<thead>
<tr>
<th>Administration Site</th>
<th>Preparation</th>
<th>Preparation</th>
<th>AUC ((\mu g \cdot h \cdot mL^{-1}))</th>
<th>(C_{\text{max}}) ((\mu g \cdot mL^{-1}))</th>
<th>(T_{\text{max}}) (h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>Vancomycin emulsion without unsaturated fatty acid</td>
<td>NC*</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Vancomycin emulsion with oleic acid</td>
<td>3.8 ± 1.0</td>
<td>1.2 ± 0.2</td>
<td>1.7 ± 0.7</td>
<td>2.7 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>6.5 ± 2.1**</td>
<td>2.3 ± 0.2</td>
<td>1.8 ± 1.3*</td>
<td>2.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>5.7 ± 1.0</td>
<td>2.0 ± 0.9</td>
<td>3.3 ± 0.8</td>
<td>3.0 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>3.7 ± 1.7</td>
<td>1.2 ± 0.5</td>
<td>2.1 ± 1.1</td>
<td>3.0 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>Vancomycin emulsion without unsaturated fatty acid</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.2</td>
<td>0.8 ± 0.9</td>
<td>0.8 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Vancomycin emulsion with oleic acid</td>
<td>8.2 ± 4.3</td>
<td>2.2 ± 0.8</td>
<td>1.5 ± 0.7</td>
<td>2.4 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>6.3 ± 4.0</td>
<td>2.0 ± 1.1</td>
<td>2.2 ± 1.3</td>
<td>3.0 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>5.6 ± 4.5</td>
<td>2.0 ± 0.9</td>
<td>1.4 ± 1.1</td>
<td>2.1 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>4.8 ± 2.8</td>
<td>1.6 ± 0.6</td>
<td>1.9 ± 0.7</td>
<td>2.7 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each value represents the mean ± SD (n = 3-7); (**p < 0.01; (*)p < 0.05.
* NC denotes not calculated.

Pharmacokinetic Parameters of VCM after Administration of Emulsion into the Colon and Rectum

Pharmacokinetic parameters of VCM following intracolonic and intrarectal administration of the emulsions are shown in Table 2. In the colon, AUC and \(C_{\text{max}}\) of the emulsions incorporating linoleic acid tended to be higher than those for oleic acid and linolenic acid were significantly higher compared with the unsaturated fatty acids. However, consistent differences that related to a degree of unsaturation were not observed with these parameters. In the rectum, there were no significant differences among the emulsions incorporating unsaturated fatty acids. In addition, obvious site differences in these parameters could not be observed. Contrary to the case of insulin, AUC values of the emulsion incorporating DHA seem to be almost the same as that of C18 unsaturated fatty acid.

Comparison of Bioavailability

To calculate the enteral bioavailability of VCM, the relationship between iv VCM dose and AUC was obtained from the results of the iv study. The relationship gave the following equation:

\[
\text{AUC} = 3.04 \times \text{dose} + 0.005 (r = 0.98, p < 0.01)
\]

Figure 4 shows the bioavailability following administration of the emulsions into the colon and rectum. In both administration sites, very low bioavailabilities were observed in the control group. In contrast, enteral bioavailabilities of VCM were clearly improved by the emulsion incorporating unsaturated fatty acids. In the colon, linoleic acid and linolenic acid showed ~40% bioavailability, which was significantly higher than that of oleic acid. This observation is consistent with previous studies. In the colon, the insulin absorption enhancement by oleic acid was relatively lower than those of polyunsaturated fatty acids. In the rectum, bioavailability was similar among various emulsions; in the range 40–50%.

In our previous study, DHA was more effective than unsaturated fatty acids with a C18 alkyl chain for improving insulin mucosal absorption.
However, this result was not observed in the case of VCM. The effectiveness of DHA on improving VCM absorption was as strong as that of oleic acids in the colon. A powerful absorption enhancement by DHA occurred only for insulin. The reason for the differing efficacies between VCM and insulin remains to be clarified. In the case of insulin, DHA might act to inhibit insulin degradation in the cytosol or hepatocytes, or act on the facilitation of receptor-mediated transport of insulin, in addition to an increase in membrane fluidity. Effects other than promoting membrane fluidity might result in the difference in ranking of drug absorption enhancement.

Effects of Fatty Acid Amount in the Emulsion on VCM Absorption Enhancement

It is thought that absorption enhancement depends on the concentration of absorption enhancers. Actually, our previous study has shown that the insulin emulsion incorporating 2% unsaturated fatty acid induced a strong hypoglycemic effect, whereas 1%unsaturated fatty acid showed only a small hypoglycemic effect. To clarify the effect of higher amounts of unsaturated fatty acid, emulsions incorporating 15% unsaturated fatty acids were prepared, and the efficacy on absorption enhancement was compared. Figures 5 and 6 show VCM plasma concentrations after colonic and rectal administration of the emulsions incorporating oleic acid and DHA, respectively. As shown in Figure 5, no obvious significant differences in the AUC of VCM were observed between the emulsions incorporating 2 and 15% oleic acid in either administration site (mean ± SE of AUC of 2 versus 15% oleic acid is 3.8 ± 0.5 versus 3.0 ± 2.0 µg · h · mL⁻¹ in the colon, and 8.2 ± 2.1 versus 7.8 ± 0.7 µg · h · mL⁻¹ in the rectum, respectively). Although AUC of the emulsion incorporating 15% DHA following colonic administration tended to be higher than that of the emulsion incorporating 2% DHA, the difference was not significant (Figure 6). In the rectum, no differences in the AUC of VCM were observed between the emulsions incorporating 2 and 15% DHA (mean ± SE of AUC of 2 versus 15% DHA is 2.2 ± 0.7 versus 4.3 ± 3.0 µg · h · mL⁻¹ in the colon, and 5.7 ± 0.8 versus 4.1 ± 1.6 µg · h · mL⁻¹ in the rectum, respectively).

It was recently suggested that the intestinal uptake of long-chain unsaturated fatty acids might be carrier-mediated. Furthermore, saturation in the capacity for absorption of EPA or DHA was suggested. It seems that a certain amount of fatty acid is required to enhance drug absorption; however, there might be a limit for uptake into the membrane. Therefore, unsaturated fatty acids might not promote drug absorption in proportion to the incorporated amount.

Effects of the Emulsion on Rₐ of Colonic and Rectal Membranes

The absorption-promoting ability of several compounds has been ascribed to their Ca²⁺-binding
ability. Chelation of Ca\(^{2+}\) may alter the structure of the tight junctions of the cell and therefore affect \(R_m\).\(^8\) It is well known that EDTA, a typical chelating agent, removes calcium from the tight junctions of the membrane to enhance paracellular permeability.\(^1\) Sodium caprate was the most effective medium-chain fatty acid and had the greatest effect on paracellular permeability, in part due to the association of calcium and fatty acid.\(^22\) As shown in Figure 7, these active controls decreased \(R_m\) rapidly. In contrast, \(R_m\) values were not changed by the emulsions incorporating various fatty acids that were maintained during the experimental period (Figure 8). The results indicate that the emulsions incorporating unsaturated fatty acid predominantly act on the transcellular pathway, and at least may not enhance VCM and insulin mucosal absorption by acting on tight junctions of the membrane. The enteral VCM bioavailability was similar, or a little lower, than the entrapment efficiency value of VCM in the emulsion. Because the emulsions may not predominantly affect the tight junctions, VCM entrapped in the emulsion might contribute to enteral absorption via the transcellular pathway.

In this study, although the concentration of unsaturated fatty acids was \(-14\) times higher than EDTA and sodium caprate, \(R_m\) values were not affected by the emulsion. Recently, Jiang et al. showed that \(\gamma\)-linolenic acid and EPA in culture

![Figure 7. Effect of EDTA and sodium caprate on tissue membrane resistance. Key: (○) colon; (O) rectum. Each value represents the mean ± SE (n = 3).](image)

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Figure 8. Effect of the emulsion incorporating unsaturated fatty acid on tissue membrane resistance. Key: (●) colon; (○) rectum. The control is the emulsion without unsaturated fatty acid. Each value represents the mean ± SE (n = 3–5).

medium increased transendothelial cell resistance and reduced paracellular permeability to large molecules via their effect on the expression of occludin.7 In our study, unsaturated fatty acid is added to the oily phase at 10%; therefore, their actions on mucous membranes might be through or with the triglyceride oily phase. These differences in the presence of fatty acids might result in the difference in effect on the \( R_e \) value. However, a more detailed study is needed to clarify the mechanism of mucosal enhancement of the emulsion incorporating polyunsaturated fatty acids.

ACKNOWLEDGMENTS

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REFERENCES


7. Jiang WG, Bryce RP, Horrobin DF, Mansel RE. 1998. Regulation of tight junction permeability and