NOTE

Lysoglyceroglycolipids Improve the Intestinal Absorption of Micellar Fucoxanthin by Caco-2 Cells

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Abstract: To improve the intestinal absorption of fucoxanthin, we evaluated the effects of dietary glyceroglycolipids on the uptake and secretion of fucoxanthin solubilized in mixed micelles by human intestinal Caco-2 cells. Although digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol suppressed fucoxanthin uptake and secretion, their lyso-types, digalactosylmonoacylglycerol and sulfoquinovosylmonoaeryl glycerol, remarkably enhanced them. Thus, some dietary glyceroglycolipids may be potential enhancers of fucoxanthin bioavailability in humans.

Key words: Caco-2, fucoxanthin, glyceroglycolipid, intestinal absorption, mixed micelles

1 INTRODUCTION

Fucoxanthin has been reported to have beneficial biological effects in cell cultures and animal models. However, the bioavailability of fucoxanthin in humans is extremely lower than that of other carotenoids such as β-carotene and lutein. The bioavailability of dietary carotenoids can be affected during the several steps required for their intestinal absorption: carotenoid release from the food matrix, dispersion as lipid emulsion, and solubilization into mixed micelles consisting of bile salts, fatty acids, monoacylglycerols, cholesterol, and phospholipids. Only the carotenoid after solubilization into mixed micelles becomes accessible to absorption by the intestinal epithelial cells and, in turn, secretion into lymph as chylomicrons. The major dietary sources of fucoxanthin are brown algae such as wakame, kombu, and hijiki. Wakame contains 0.6 mg of fucoxanthin per serving (25 g of steamed preparation). In a study of human subjects with a daily intake of wakame containing 6.1 mg fucoxanthin and 0.2 mg β-carotene contents for 1 week, the plasma concentration of fucoxanthin (a fucoxanthin hydrolysate, Fig. 1) was under the quantification limit (1 nM), while that of β-carotene was on the order of a few μM. The solubilization of fucoxanthin from wakame...
was comparable to that of \( \beta \)-carotene in an in vitro experiment\(^{3}\), suggesting the solubilization did not cause the low bioavailability of fucoxanthin. After ingestion of kombu extract (containing 31 mg fucoxanthin), the human plasma concentration of fucoxanthinol was 44.2 nM\(^4\), but it was lower than those of \( \beta \)-carotene\(^3\), lutein\(^3\), and \( \beta \)-carotene 5,6-epoxide\(^5\). Although the mechanism underlying the low bioavailability of fucoxanthin has not yet been clarified, it could be caused by the steps following the solubilization. Only a portion of the solubilized carotenoids is thought to be absorbed by the cells\(^6\). An increase in intestinal absorption is thus a critical factor in increasing the carotenoid bioavailability. We recently found that lypo-lipids derived from dietary glyceroglycolipids (GLs) such as digalactosyldiacylglycerol (DGDG) and sulfogalactosyldiacylglycerol (SQDG) enhanced the uptake of carotenoids such as \( \beta \)-carotene and lutein solubilized in mixed micelles by human intestinal Caco-2 cells\(^7\). In the present study, we evaluated the effect of glyceroglycolipids not only on the uptake but also on the transport of fucoxanthin through Caco-2 monolayers in the Transwell plates.

2 EXPERIMENTAL

2.1 Materials

Fucoxanthin and fucoxanthinol were prepared as previously reported\(^2\). Monogalactosyldiacylglycerol (MGD)\(^2\), DGDG, and SQDG were purchased from Larodan Fine Chemicals (Malmö, Sweden). Their lypo-glycerolipids (lyoGLs), monogalactosylmonooacylglycerol (MGMG), digalactosylmonooacylglycerol (DGMG), and sulfogalactosylmonooacylglycerol (SQMG), were prepared as previously reported\(^7\). Their major fatty acids (mol%) were 16:0 (4.3), 16:1 (21.4), and 18:3 (67.5) for MGDG, 16:0 (8.0), 16:3 (4.0), and 18:3 (80.1) for DGDG, 16:0 (36.1), 16:1 (3.6), and 18:3 (53.6) for SQDG, 16:3 (91.7), 18:2 (1.1), and 18:3 (4.9) for MGMG, 16:0 (11.2), 16:3 (3.8), and 18:3 (79.1) for DGMG, and 16:0 (38.9), 16:1 (12.7), and 18:3 (41.8) for SQMG. Based on the fatty acid compositions, we calculated the average molecular weight, and then determined the molar amounts\(^7\).

2.2 Cell culture

The experiment of carotenoid absorption (uptake and transport) by Caco-2 cells (American Type Culture Collection, Rockville, MD) was carried out as described previously\(^8\). In brief, cells were seeded in polycarbonate Transwell® 6-well plates (Corning Inc., Corning, NY) at \( 3 \times 10^5 \) cells/well, and cultured for 20-23 days at 37°C in a humidified atmosphere of 5% CO\(_2\) in air. Transepithelial electrical resistance (TEER) was measured by a voltmeter equipped with a chostick-type electrode (EVOMX and STX2 electrode; World Precision Instruments, Sarasota, FL) on the day before the experiment. The obtained TEER values were approximately 4700Ω, indicating the formation of tight monolayers.

2.3 Preparation of mixed micelles containing fucoxanthin

Mixed micelles were prepared as described previously\(^7\). In brief, all the mixed micelle components and fucoxanthin dissolved in appropriate solvents were transferred to glass test tubes. The solvent was evaporated under a stream of argon and further dried in a centrifugal evaporator. The dry matter was dispersed into FBS-free DMEM (containing phenol red) and the resulting clear solution was passed through a 0.2-\( \mu \)m filter to remove unmicellized fucoxanthin. The mixed micelles consisted of 1.5 \( \mu \)M fucoxanthin, 2 \( \mu \)M sodium taurocholate, 33.3 \( \mu \)M oleic acid, 100 \( \mu \)M monoolein, 0 or 50 \( \mu \)M GLs/lysoGLs, and 0.15 \( \mu \)M \( \alpha \)-tocopherol as an antioxidant. The mixed micelles and FBS-containing DMEM without phenol red were added to the apical side and the basolateral side of the tight monolayers in Transwell plates, respectively, after the both sides were washed twice with FBS-free DMEM without phenol red. Then, the tight monolayers were incubated for 4 h at 37°C.

2.4 Evaluation of the uptake of fucoxanthin and fucoxanthinol by Caco-2 cells from micelles and the transport to the basolateral side

Immediately after the incubation, the basolateral medium was collected to evaluate the transport of fucoxanthin and fucoxanthinol through the monolayers. The cells were washed twice with Hank's balanced salt solution and then collected as described previously\(^3\) to measure the cellular uptake of fucoxanthin and fucoxanthinol. Extracts of fucoxanthin and fucoxanthinol from the basolateral medium or the cells were dissolved in DMSO/methanol/water (2:7.1, \( \nu/\nu/\nu \)) . The apical medium was collected to evaluate residual micellar fucoxanthin and fucoxanthinol, and then diluted 9-fold with DMSO/methanol (2:7, \( \nu/\nu \)). These samples were analyzed by HPLC as described previously\(^7\). The solvent system consisted of acetonitrile/methanol/water (75:15:10, \( \nu/\nu/\nu \)), containing 0.1% ammonium acetate. Cellular protein contents were determined as described previously\(^8\).

Phenol red has been used as a marker for paracellular transport\(^9\), which is the permeation through the intercellular junction from the apical side to the basolateral side. After the incubation in the present study, phenol red concentration in the basolateral medium was measured under an alkaline condition at 560 nm with a microplate reader (Tecan Group, Mannedorf, Switzerland). All experiments were done under dim yellow light to minimize isomerization and degradation of fucoxanthin by light irradiation.

2.5 Statistical analysis

The data were tested by one-way ANOVA, followed by
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3 RESULTS

3.1 Effect of glyceroglycolipids on fucoxanthin absorption

Mixed micelles not containing GLs/lysoGLs were used as controls in the experiment, and are indicated as NoGL micelles.

The upper panels (A and B) in Fig. 2 show the combined amounts of fucoxanthin and fucoxanthinol taken up in Caco-2 cells after 4 h of incubation. DGDG and SQDG suppressed the uptake to about 50% of that in the NoGL. DGMG and SQMG significantly enhanced the uptake. MGDG and MGMG did not affect the uptake. The lower panels (C and D) in Fig. 2 show the combined amounts of fucoxanthin and fucoxanthinol transported into the basolateral side. The effects of GLs/lysoGLs on the transport were almost consistent with those of GLs/lysoGLs on the uptake, except in the case of MGDG. Phenol red was not detected in the basolateral medium.

3.2 The residual amounts of the micellar carotenoids

Table 1 shows the combined residual amounts of micellar fucoxanthin and fucoxanthinol at the end of the incubation. The amounts in NoGL micelles were significantly higher than those in other micelles, except for NoGL vs. MGMG.

4 DISCUSSION

We previously reported that phospholipids markedly affected the absorption (uptake and transport) of β-carotene by Caco-2 cells. Lysophosphatidylcholine (lysoPC) also enhanced the absorption of fucoxanthin (data not shown). In the present study, we found that DGMG and SQMG significantly enhanced the uptake of fucoxanthin. Most of fucoxanthin was hydrolyzed to fucoxanthinol during the uptake by Caco-2 cells, as described previously. Fucoxanthin has a much higher polarity than β-carotene and lutein (Fig. 1). This result, together with our previous findings, suggests that the uptake of fucoxanthin by Caco-2 cells is influenced by the presence of GLs/lysoGLs.

Table 1 The combined residual amounts of the micellar fucoxanthin and fucoxanthinol after incubation.

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<th>Diacyl (μM)</th>
<th>Monoacyl (μM)</th>
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<td>NoGL</td>
<td>1.03 (0.88 + 0.15) ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91 (0.78 + 0.13) ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>MGDG</td>
<td>0.88 (0.79 + 0.08) ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.03 (0.88 + 0.15) ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>DGDG</td>
<td>0.85 (0.83 + 0.02) ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 (0.48 + 0.17) ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SQDG</td>
<td>0.65 (0.62 + 0.03) ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.52 (0.39 + 0.13) ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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Caco-2 cells were treated with fucoxanthin solubilized in mixed micelles with or without glyceroglycolipids for 4 h. Micellar fucoxanthin (Fuco) and fucoxanthinol (FuOH) in the recovered medium were analyzed by HPLC, and expressed as the combined amounts (Fuco + FuOH). Data represent the means ± SD values of four wells of a single experiment. The values not sharing a common letter are significantly different by the Tukey–Kramer test (p < 0.05).
on the uptake of β-carotene and lutein, suggests that these lysoGLs enhance the absorption of various carotenoids with different polarities. We previously reported that the integrity of the intercellular barrier formed by cell-to-cell/cell-matrix adhesion participated in the mechanism underlying the effect of lyso-lipids on the carotenoid uptake by Caco-2 cells. In brief, DGMG and SQMG would enhance carotenoid uptake by decreasing the intercellular barrier integrity through the removal of cellular cholesterol, and this applies to carotenoids of a wide polarity range.

The concentration of micellar carotenoids in the incubation media may be decreased not only by cellular uptake but also by oxidative degradation and insolubilization of carotenoids due to the cellular uptake of other micellar components such as fatty acids and monoolein during the incubation. The decrease in micellar carotenoid might reduce the transfer of carotenoid from micelles into the cells, because only micellar carotenoid can be taken up by Caco-2 cells. However, in the present study, there was no association between the amount of residual micellar carotenoid and the uptake. In addition, we previously found that the concentration of micellar carotenoid at the end of the incubation was not an important factor modulating the uptake.

DGMG and SQMG enhanced not only the uptake but also the transport of carotenoid into the basolateral side of the Transwell, which simulates the transport into lymph. There are two possible transport pathways: paracellular and tran-

5 CONCLUSION

Lyso-lipids such as DGMG and SQMG could improve the intestinal cell absorption of fucoxanthin. The present study suggests that dietary glyceroglycolipids have the potential to enhance fucoxanthin bioavailability through an increase in the intestinal uptake and secretion to lymph.

![Proposed model for the pathway of carotenoid absorption (uptake and secretion).](image-url)

Caco-2 cells were treated with NoGL micelles (A) or lyso-lipid micelles (B). Lyso-lipids enhance the cellular uptake of carotenoid by reducing the integrity of adhesion through the removal of cellular cholesterol, and then the secretion of cellular carotenoid to the basolateral side by increasing the expression of apolipoproteins.
ACKNOWLEDGMENTS

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References