Docosahexaenoic acid and eicosapentaenoic acid-enriched phosphatidylcholine liposomes enhance the permeability, transportation and uptake of phospholipids in Caco-2 cells

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Abstract

The influence of docosahexaenoic acid (DHA)- and eicosapentaenoic acid (EPA)-enriched phosphatidylcholine (PC) on the permeability, transport and uptake of phospholipids was evaluated in Caco-2 cells. The cells were grown on permeable polycarbonate transwell filters, thus allowing separate access to the apical and basolateral chambers. The monolayers of the cells were used to measure lucifer yellow permeability and transepithelial electrical resistance (TEER). Transcellular transportation of diphenylhexatriene (DPH) labeled-PC small unilamellar vesicles (SUV) from the apical to basolateral chamber, and uptake of the same SUV was monitored in the cell monolayers. Cell-membrane perturbation was evaluated to measure the release of lactate dehydrogenase and to determine the cell viability with sodium 2-(4-iodophenyl)-3-(4- nitrophenyl)- 5- (2, 4-disulfophenyl) -2*H*-tetrazolium dye reduction assay. The lucifer yellow flux was 1.0 and 1.5 nmol/ h/ cm^2 with 50 μM PC, and 17.0 and 23.0 nmol/ h/ cm^2 with 100 μM PC when monolayers of Caco-2 cells were treated with DHA- and EPA-enriched PC, respectively. TEER decreased to 24 and 27% with 50 and 100 μM DHA-enriched PC, and to 25 and 30% with 50 and 100 μM EPA-enriched PC, respectively. Our results show that DHA- and EPA-enriched PC increases tight junction permeability across the Caco-2 cell monolayer whereas soy PC has no effect on tight junction permeability. Transportation and uptake of DHA- and EPA-enriched PC SUV differed significantly ($P<0.01$) from those of soy PC SUV at all doses. We found that PC SUV transported across Caco-2 monolayer and was taken up by Caco-2 cells with very slight injury of the cell membrane up to 100 μM PC. Lactate dehydrogenase release and cell viability did not differ significantly between the treatment and control, emphasizing that injury was minimal. Our results suggest that DHA- and EPA-enriched PC enhance the permeability, transport and uptake of PC SUV across monolayers of Caco-2 cells.

Key words: docosahexaenoic acid, eicosapentaenoic acid, tight junction, permeability, Caco-2 cell line

Introduction

Humans can synthesize all fatty acids needed for various biological activities except the ω-3 and ω-6 fatty acids. The ω-3 fatty acids alter the intestinal morphology and increase nutrient permeability and transportation [1,2]. Additionally, the ω-3 fatty acids are widely used for treatment of colon cancer through inhibiting cyclooxygenase 2, increasing apoptotic activity, reducing angiogenesis, and activating protein kinase C [3,4]. However, there are few studies on the effects of the ω-3 and ω-6 fatty acids on intestinal permeability, and the transport or uptake of these fatty acids in liposome form into intestinal cells. The gastrointestinal epithelium normally functions as a selective barrier that permits the absorption of nutrients, electrolytes, and water, but restricts the passage from the lumen into the systemic circulation of larger potentially toxic compounds. This characteristic of the intestinal mucosa, which is referred to as "selective permeability", appears to be mediated by tight junctions. It has also been proposed that an abnormal increase of tight junction permeability allows increased intestinal penetration of toxic antigens, which can lead to intestinal diseases [5].

Human colon carcinoma Caco-2 cells undergo spontaneous *in vitro* differentiation when they are grown on transwell filters. Transwell filters allow independent manipulation of the apical and basolateral media of the monolayer [6]. They express typical structural characteristics and functional properties of small intestinal enterocytes [7,8]. They have microvillar brush border membranes, well-developed

tight junctions and display polarity when fully differentiated [6-9]. Among the *in vitro* systems employed to predict bioavailability of drugs in humans, the Caco-2 monolayer model has been used widely in studies concerning both active and passive transport of drug molecules, nutrients and other chemicals [6,10]. This model is also well known with respect to intestinal lipid absorption, transport and metabolism [11].

Phospholipid liposomes have the advantages of biodegradability, lack of antigenicity, protection of encapsulated drugs against degradative enzymes and reduction of drug toxicity [12]. Our studies of the ω-3 and ω-6-enriched phospholpids, suggest they may alter the physical structure of phospholipid bilayer cell membrane, producing changes in membrane environment and altering the cell response for permeability, transportation and uptake. Long chain fatty acid (LCFA) uptake has been examined a great deal *in vivo* with radio-labeled lipids [13,14], but the uptake of phospholipids has not been studied *in vitro* using fluorescent probes. In the present study, we investigated the permeability effects of DHA- and EPA-enriched PC, and compared with soy PC, and observed the transportation and uptake of PC small unilamellar vesicles (SUV) labeled with fluorescent probes in Caco-2 cells.

Materials and methods

Materials

Human colon carcinoma Caco-2 cell lines were obtained from American Type Culture Collection (Rockville, CT, USA). Minimum essential medium (MEM), Dulbecco's modified eagle medium (DMEM) and penicillin streptomycin were obtained from GIBCO (Grand Island, NY, USA). Fetal bovine serum (FBS), fetal calf serum (FCS) and L-glutamine were obtained from ICN Biomedicals Inc. (Costa Mesa, CA, USA). Morpholinoethanesulfonic acid (MES) was provided by Sigma Chemicals Company (St. Louis, MO, USA). Lucifer yellow CH lithium salt was purchased from Molecular Probe Inc. (Eugene, OR, USA). Sodium 2-(4-iodophenyl)-3-(4- nitrophenyl)- 5- (2, 4-disulfophenyl) -2*H*-tetrazolium (WST-1), 1-methoxy-5-methyphenazinium methylsulfate (1-methoxy PMS) and 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) were obtained from Dojindo Laboratories (Kumamoto, Japan). 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Tokyo Kasei (Tokyo, Japan). Squid meal phospholipids were generous gifts from Nippon Chemical and Feed Co. Ltd., (Hakodate, Hokkaido, Japan). Soy phosphatidylcholine (PC) was purchased from NOF Corporation (Tokyo, Japan).

Cell culture

Caco-2 cells were grown in MEM with 26.2 mM sodium bicarbonate, 100 IU/ml penicillin, 100 µg/ml streptomycin, 1% nonessential amino acids, and 10% heat-incubated FBS in a 95% air-5% CO_2 atmosphere at 37^0C. The cells were grown in 25-cm^2 flasks, and subcultured at every three-day intervals on a routine basis at a

concentration of 5 x 10^4 cells/ml.

Extraction of starfish phospholipids

Total lipid was extracted from starfish following the method of Bligh and Dyer [15]. The extracted lipids were applied to a silica gel column chromatography and eluted with chloroform to afford simple lipids. The column was flushed with methanol to afford phospholipids. Silica gel thin layer chromatography (TLC) plate (Darmstadt, Merck, Germany) was developed with chloroform/ methanol/ water (65:25:4, v/v/v) for phospholipid analysis.

Separation of PC from soy, squid meal and starfish phospholipids

PC from soy, squid meal and starfish phospholipids was separated on TLC according to the method of our previous report [16]. Briefly, phospholipids were loaded on 20 X 20 cm^2 preparative glass plates coated with silica gel 60 (Darmstadt, Merck, Germany) at a concentration of 100 mg phospholipid/ plate. The PC was separated with a developer consisting of chloroform/ methanol/ water (65:25:4, v/v/v). The PC band was monitored on the TLC using Dittmer solution. The PC band was visualized under UV light at 254 or 366 nm in a dark room and compared with an authentic standard. The PC-containing band was scraped off and immediately eluted with methanol. Methanol elutes were evaporated, dissolved in a chloroform/ methanol/

water (10:5:3, v/v/v) mixture and placed in a separating funnel to remove the silica gel. The separating funnel was kept at 4-5^{0}C overnight, and then the chloroform layer was collected and evaporated at approximately 25^{0}C to obtain the PC.

Fatty acid composition of soy, squid meal and starfish PC

Individual methyl esters were derived from the PC sample following the method of Lepage and Roy [17] with slight modification. The dried samples were dissolved in 5% methanolic-HCl. The mixture was shaken, kept at 80^{0}C for 2 h and then 2 ml water and 3 ml hexane were added. The n-hexane layer was collected, concentrated and subjected to gas chromatographic analysis with a Hitachi 163 gas chromatograph (Hitachi Co. Ltd., Ibaraki, Japan) connected with a 0.5 μm PEG-20M liquid phase-coated 40 m x 1.2 mm ϕ G-300 column (Chemicals Evaluation and Research Institute, Saitama, Japan) that was equipped with flame ionization detection. The temperatures of the column, detector and injector were 170, 250 and 240^{0}C, respectively. The fatty acids were identified by comparing the peak retention times with authentic standards (St. Louis, MO, USA) following the method of Takahashi *et al.* [18].

Transportation of lucifer yellow

For transport studies, the cells were plated at a density of 2 x 10^5 cells/ cm^2 onto a 12

mm polycarbonate transwell filter with 0.4-μm pores and a surface area of 0.6 cm^2 (Millipore Corporation Ltd., Bedford, MA, USA). The filter was placed in 24-well cell culture plates. The cells were grown in DMEM with 25 mM glucose, 4 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1% nonessential amino acids, and 10% heat-incubated FCS in a 95% air-5% CO_2 atmosphere at 37^0C. These cells were given fresh DMEM at three-day intervals until use. Cells were grown for 20 days (postconfluence) before the experiments. For this series of studies, cell passage numbers from 25 to 35 were used. Before each experiment, cells were washed twice with PBS and pre-equilibrated for 10 min in Hanks balanced salt solution (HBSS: 137 mM NaCl, 5.36 mM KCl, 0.44 mM KH_2PO_4, 1.00 mM $CaCl_2$, 1.00 mM $MgCl_2$, 5.60 mM glucose, 0.30 mM Na_2HPO_4) containing 10 mM MES at pH 6.0 in the apical (AP) chamber and HBSS containing 10 mM HEPES at pH 7.4 in the basolateral (BL) chamber. Lucifer yellow CH (LY) was dissolved in the HBSS. Soy, squid meal, and starfish PC at different concentrations (25, 50 and 100 μM) and 100 μM LY were applied to the AP chamber of the Caco-2 cell monolayer. The pH conditions were chosen in order to reproduce the physiological pH gradient existing *in vivo* across the small intestinal mucosa. After incubation at 37^0 C for 1 h, the HBSS in the BL chamber was collected, and the concentration of LY was determined by measuring the fluorescent intensity with a Hitachi spectrophotofluoremeter (Hitachi Co. Ltd., Ibaraki, Japan). The excitation and emission wavelengths used were 430 and 540 nm, respectively.

Preparation of liposomes

Squid meal PC (50 mg) dissolved in chloroform was collected in a flask and dried to a thin film by rotation under reduced pressure at 30^0 C. Afterwards, the lipid film was maintained at low pressure by connection to a vacuum rotary pump for 3 h. An aliquot of DPH (DPH: squid PC = 1: 100, w/w) in tetrahydrofuran was added to the flask as a lipid-fluorescent probe. The mixtures were evaporated for 1 h in the dark under N_2, resuspended in 10 ml HBSS buffer (pH 6.0) and stirred for 30 min at 1000 rpm. Then 5 ml HBSS was added and the mixture was stirred for 20 min at 700 rpm. A total of 10 ml of HBSS was added to make a final PC concentration of 200 μM. The multilamellar vesicles (MLV) were sonicated for 30 min (15 x 1 min of sonication and 1 min of no sonication) in the dark under N_2 to prepare SUV. The liposome suspension was centrifuged at 8000 x g for 10 min to remove debris. The supernatant was removed and centrifuged again at 15000 x g for 5 min. The tubes were sliced into 1 ml section from the top and sterile filtered through a 0.22 μm millipore filter. Each sample was diluted with HBSS to form final lipid concentrations of 25, 50 and 100 μM. The size distribution of liposomes was determined using a dynamic light scattering particle size analyzer LB-500 (HORIBA, Japan).

Transportation and uptake of liposomes

For transportation and uptake studies, cells were plated at a density of 2 x 10^5 cells/

cm^2 onto a 30 mm polycarbonate transwell filter with 0.4-μm pores and a surface area of 4.2 cm^2. The filter was placed in 6-well cell culture plates. The cells were grown as described for the experiment of LY transportation. Before each experiment, cells were washed twice with PBS and pre-equilibrated for 10 min with HBSS containing 10 mM MES at pH 6.0 in the AP chamber and HBSS containing 10 mM HEPES at pH 7.4 in the BL chamber. Caco-2 cells were treated for 5 h with increasing concentrations of DPH-labeled liposomes in HBSS at pH 6.0 in the AP chamber, while the BL chamber contained HBSS at pH 7.4. After treatment, the treating solution was withdrawn and the cells were washed three times with PBS. HBSS in the BL chamber was collected and by adding 0.24 mM Triton X –100, transported liposomes were lysed. Cells of each transwell filter were trypsinized, collected and lysed. Fluorescent intensity was determined with a Hitachi specterophotofluoremeter. The excitation and emission wavelengths used were 360 and 430 nm, respectively.

TEER measurement

The transepithelial electrical resistance (TEER) was measured using a Millicell$^{(R)}$ ERS (Millipore Co., Bedford, MA, USA), to ensure the integrity of the monolayers formed on the filter. The average TEER was 952 ± 54.3 Ω cm^2. TEER measurements were started during the pre-equilibration time in HBSS and continued during the treatment. Values were recorded every 20 min over the experimental period. Control filters were maintained with only LY for 1 h for permeability experiments; or only HBSS for 5 h for

transportation and uptake experiments, displayed a stable baseline of TEER values (data not shown). TEER data was expressed as a percentage of the initial values at each time point.

LDH release assay

Measurements were performed using a LDH-Cytotoxicity Assay Kit (BioVision Research Products, Mountain View, CA, USA) following the instructions provided by the manufacturer with slight modifications. Briefly, 200 μl of DMEM containing 1% FBS was collected from the AP chamber during different experimental periods, placed into a 96 well plate, incubated for 20 min in an incubator at 37^0 C and centrifuged for 20 min at 250 x g. A total of 100 μl of the supernatant was transferred into corresponding wells. A total of 100 μl of the reaction mixtures was added to each well and incubated for 30 min at room temperature in the dark. Absorbance of all samples was measured at 490-650 nm using a precision microplate reader (Molecular Devices, Sunnyvale, CA, USA).

WST-1 dye reduction assay

Treated Caco-2 cells were collected from the transwell filter and taken into 96 well plates at 1 x 10^4 cells/ml in 200 μl growth medium per well. WST-1 (3.3 mg/ml PBS including 7% 1-methoxy PMS) was added at 20 μl in each well containing 200 μl

medium with cells, and plates were incubated (3h, 37^{0}C) in a 5% CO_2-humidified incubator. Absorbance was measured at 450-650 nm on a precision microplate reader. Cell viability was determined at 24 h following a WST-1 dye reduction assay.

Statistical analysis

Statistical analysis was performed using the statistical software package SPSS. Data was expressed as means ± standard deviations (SD). ANOVA was performed using SPSS. To identify significant differences, Duncan's multiple range test was used to compare each group, and the resulting *P* values for each group indicated in the figures.

Results

Fatty acids composition

Fatty acid compositions of PC isolated from soy, squid meal and starfish phospholipids are shown in Table 1. The major fatty acids were 16:0, 18:1 and 18:2 in soy PC; 16:0, 20:5 and 22:6 in squid meal PC, and 18:0, 20:1, 20:5 and 22:6 in starfish PC.

Liposome size distribution

The mean particle sizes of DPH-probe liposomes of soy, squid and starfish PC were 95.08 ± 65.91, 90.1 ± 59.2 and 98.32 ± 64.81 nm; each ranged from 34.2 to 241.2, 29.1 to 225.8 and 31.2 to 245.5 nm, respectively. Particles smaller than 100 nm composed 64.32, 67.89 and 65.23% of the liposome suspension for soy, squid and starfish PC, respectively (Fig. 1).

DHA- and EPA-enriched PC decreased the TEER of Caco-2 cell monolayer

Soy PC did not decrease TEER during the experimental period (Fig. 2A). However, treatment of Caco-2 monolayers with 25, 50 and 100 μM squid meal and starfish PC resulted in decreases in TEER by 21, 24 and 27% for squid meal PC, and 9, 25 and 30% for starfish PC respectively, (Figs. 2B and 2C) in the 20-min treatment. The TEER of control treated monolayers (LY alone) did not change over the treatment time, remaining at approx. 100% of the initial baseline (time=0) TEER values.

DHA- and EPA-enriched PC increased tight junction permeability

The LY flux across the Caco-2 cell monolayers at a pH of 6.0 is depicted in Fig. 3 after incubation with DHA- and EPA-enriched PC. The paracellular permeation of LY was increased for the 100 μM PC, but the increase was much smaller for the 50 μM PC.

The LY flux at 100 μM differed significantly from that at 50 μM. However, LY flux was not found in soy PC up to 100 μM.

LDH release and cell viability

As shown in Fig. 4, there were no significant releases of LDH from the Caco-2 cells into the extracellular fraction during incubating with up to 100 μM PC for 3, 12, and 24 h. All three types of phospholipids showed similar results. In addition, analysis of plasma membrane integrity using a WST-1 dye reduction assay revealed that cell viability was not affected significantly after 24 h exposure of up to 100 μM PC liposomes (Fig. 5).

Transportation and uptake of fluorescent probe liposomes in Caco-2 cells

The liposome penetration through the Caco-2 cell monolayers was determined by measuring the fluorescent intensity. The TEER of the Caco-2 cell lines was found to be 850-1140 ohm/ cm^2 in HBSS. Transportation of PC liposomes from apical to basolateral layers was 10, 23 and 56 nmol/ 5h/ cm^2 for soy PC; 60, 154 and 236 nmol/ 5h/ cm^2 for squid PC and 43, 143 and 201 nmol/ 5h/ cm^2 for starfish PC at the doses of 25, 50 and 100 μM PC, respectively (Fig. 6A). Uptake of PC liposomes by Caco-2 cells was 100, 453 and 787 nmol/ 5h/ cm^2 for soy PC; 1051, 1583 and 2293 nmol/ 5h/ cm^2 for squid PC and 804, 1332 and 1558 nmol/ 5h/ cm^2 for starfish PC with 25, 50

and 100 μM PC, respectively (Fig. 6B). Transportation and uptake of squid and starfish PC liposomes was significantly higher in comparison to soy PC liposomes. Our results show that more than 20 % of added fluorescent intensity was recovered from the cells. During incubation however, some of the fluorescent probe liposomes stuck to the filter membrane and plastic wall. The fluorescent intensity decreased by about 10% after an incubation period of 5 h.

Discussion

The tight junctions (TJs) between epithelial cells are thought to work as barriers to the diffusion of some molecules between apical and basolateral domains of the plasma membrane and to seal neighboring cells together [19]. These junctions control important physiological functions in human body. This is the first report on the effect of soy, and DHA- and EPA-enriched PC on TJs. However, a few studies have examined on the effects of LCFA on TJs [20-23]. In the present study, DHA- and EPA-enriched PC decreased the TEER values and increased the paracellular permeability of LY within 20 min. Later, TEER values started to increase, reducing the paracellular permeability, indicating that TJ protein synthesis was not required. This TEER-reducing effect might have been due to DHA and EPA. TJs are composed of a transmembrane protein (occludin) and sub-membranous anchorage protein (ZO-1). Occludins that are transmembrane proteins bind to each other and form TJs in association with the proper anchorage proteins. An increased level of occludin will

facilitate the formation of TJs. Reduction of levels and distribution of occludin to the junction area has been found to be associated with damaged paracellular permeability [24]. Jiang *et al.* [19] demonstrated that DHA lacking LCFA decreased the level of occludin on the TJs. In addition, LCFA has been shown to influence TJs permeability by modifying either cyclooxygenase or lipooxygenase (20) or occludin localization (22). Our results also revealed that highly polyunsaturated DHA- and EPA-enriched PC decreased the TEER, whereas TEER did not decrease with less unsaturated soy PC.

LDH release in all three groups was both dose- and time-dependent. Levels of cell membrane damage for soy, and DHA- and EPA-enriched PC at 100 μM PC were similar and slight. For doses of up to 100 μM PC for 24 h, cell viability was not affected. This indicates the damage to the plasma membrane is slight.

In the present experiment, it is thought that particles smaller than 100 nm were transported over the Caco-2 monolayer. MacKay *et al.* [25] investigated drug delivery by fluorescent probe liposomes in rat brains. Particles larger than 200 nm in diameter did not penetrate as much as 80 nm liposomes. Additionally, it was also found that the amount of liposomal particles penetrating the mucous layer increased when the size of liposomes was reduced to 100 nm [26]. Moreover, Ho *et al.* [27] reported that when the size of the micelle is reduced, the micelle number will increase in a fixed concentration of LCFA. In the current study, the number of SUV was high at a fixed

concentration of PC.

The results indicate that the DPH-labeled SUV was transported from AP to BL and was uptaken into Caco-2 cells within 5 h. It has been reported that Caco-2 monolayers are flat compared to the extensively folded human jejunum [28]. Transport of some drugs across the Caco-2 monolayers is 30- to 80-fold slower than across the human jejunum [29]. Caco-2 cells express both liver and intestinal fatty acid-binding proteins that may play important roles in trafficking LCFA intracellularly [30]. There are two mechanisms that have been proposed for LCFA transport across the AP membrane of the enterocyte: passive diffusion through the lipid bilayer [31] and carrier-mediated transport [21, 32]. As lipophilic compounds permeate across a cellular barrier, a fraction of the solute is retained by the barrier. Krishna *et al.* [33] reported that the Caco-2 cell permeability for lipophilic molecules was as high as 54%. PC liposomes are lipophilic and hydrophilic because phospholipids are both fat loving due to the fatty acids and water loving on account of phosphorus [34]. The Acyl chain composition of phospholipids can greatly affect membrane permeability. In general, there is a direct relationship between the level of unsaturation and the intensity of membrane permeability. In the present study, transportation and uptake of squid and starfish PC was significantly higher than soy PC, as the soy PC was less unsaturated.

Liposomes can provide a protective layer against drug molecules which if administered orally are of low permeability, and unstable in the presence of enzymes

or at the pH of the gastric medium. Iwanaga *et al.* [35] showed that drug molecules in liposome form could penetrate through cell membranes effectively. In general, the main routes of drug penetration in the intestinal membrane are defined as the paracellular and transcellular routes [36]. LY is known to pass through the paracellular transport route [37]. We suggest that liposomes might penetrate through the transcellular route. Recently, we reported that DHA- and EPA-enriched PC liposomes enhanced differentiation and apoptosis of Caco-2 cells with sodium butyrate. For treatment of the human colon carcinoma (Caco-2 cells), we used a MLV of PC and phosphatidylserine (PS) [16]. We assumed that MLV did not enter the cells, rather only signals reached the cells. Cancer research might benefit more from SUV than from MLV. Furthermore, SUV might be useful for delivery of drugs that have low permeability and are unstable (e.g. insulin) even though the amount of encapsulation is low.

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Figure Legends

Fig 1. Particle size distribution of DPH-labeled small unilamellar soy PC (A), squid PC (B) and starfish PC (C) liposomes. The size distribution of liposomes was determined by a dynamic light scattering particle size analyzer LB-500 (HORIBA, Japan).

Fig. 2. Effect of soy PC (A), squid meal PC (B) and starfish PC (C) on TEER of Caco-2 cells grown on filter. Time-course of TEER variation over a 1 h treatment period. TEER values were calculated as a percentage of the initial TEER values at each time point. The data are presented as ± SD (n=4). Asterisks indicate significant difference from control (*P<0.01).

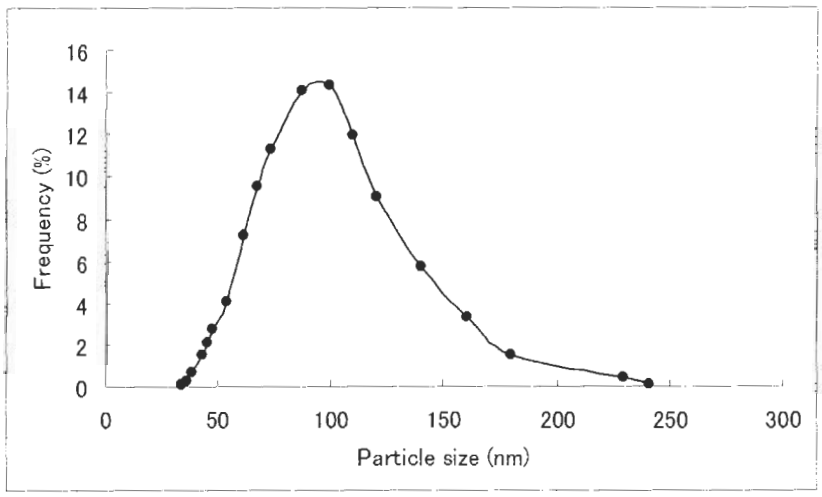
Fig. 3. Lucifer yellow flux of a cultured Caco-2 monolayer. Lucifer yellow (100 μM) was added to the apical chamber and incubated for 1 h. LY was measured by fluorescence intensity at excitation and emission wavelengths of 439 and 540 nm, respectively. Results are means ± SD (n=5). Asterisks indicate significant differences from 50 μM (*P<0.01).

Fig. 4. LDH release in the culture medium by Caco-2 cells due to the effect of soy (A), squid meal PC (B) starfish PC (C) during different treatment periods. Caco-2 cells grown on the filter inserts were treated with different concentrations of PC. Percent LDH release was measured by kit. Results are means ± SD (n=5).

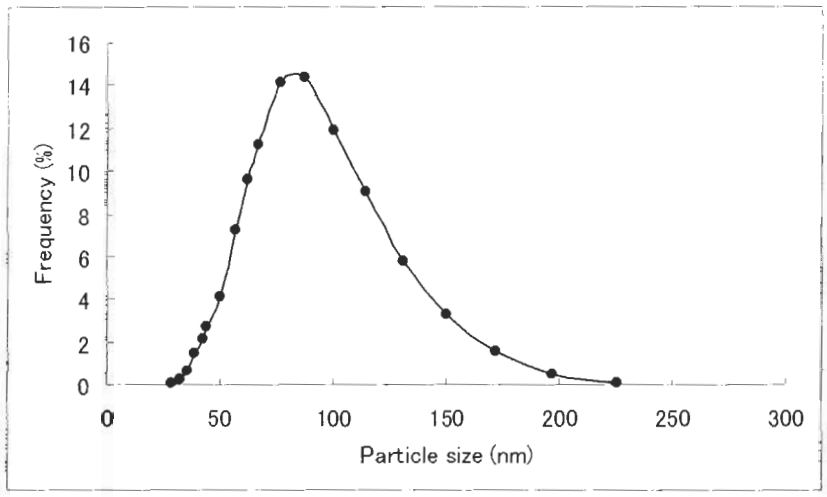
Fig. 5. Cell viability after 24 h incubation with different concentrations of soy, squid and starfish PC. PC liposomes were added to the apical solution, and incubated for 24 h. After 24 h incubation, cells were collected and assayed with WST-1 dye. Results are means ± SD (n=8).

Fig. 6. Dose-dependent transport (A) and uptake (B) of PC liposomes across a Caco-2 monolayer. Different concentrations of DPH-labeled PC liposomes were added to the apical solution and transport of DPH-labeled PC liposomes in basolateral solution and, uptake in Caco-2 cells were measured by fluorescent spectrophotometer after 5 h incubation. Fluorescent intensity was measured at

excitation and emission wavelengths of 360 nm and 430 nm respectively. Results are means ± SD (n=5). Asterisks indicate significant differences from soy PC (*P<0.01).



(A)



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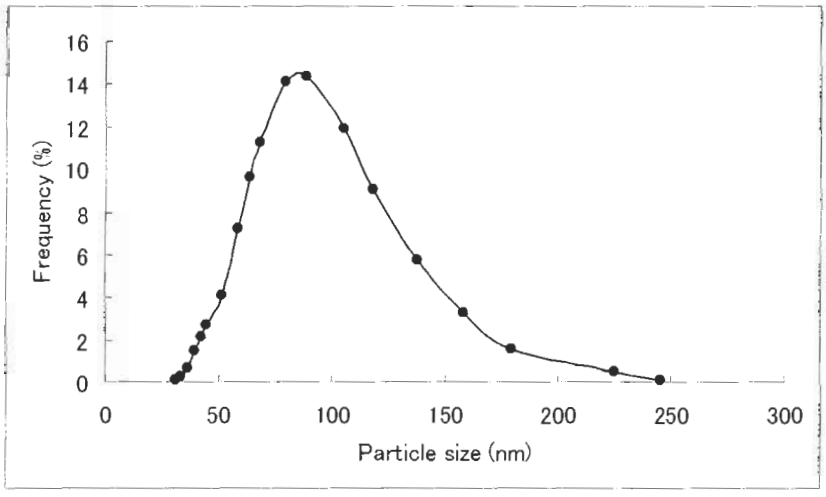

(C)

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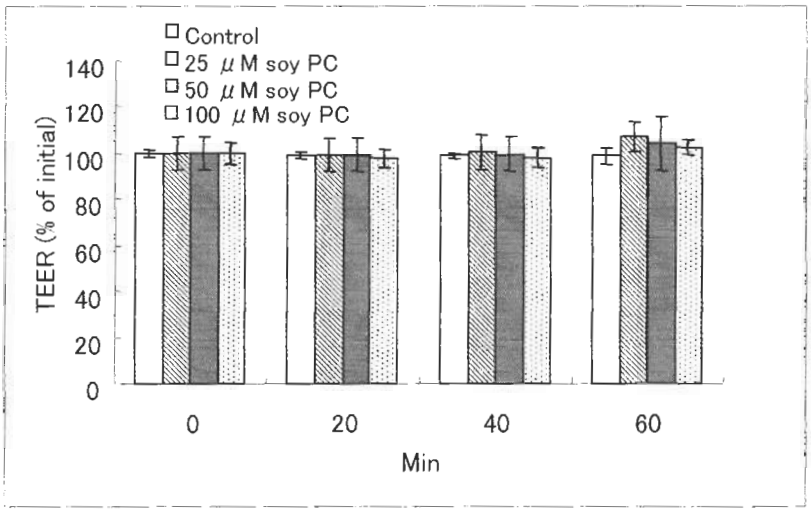
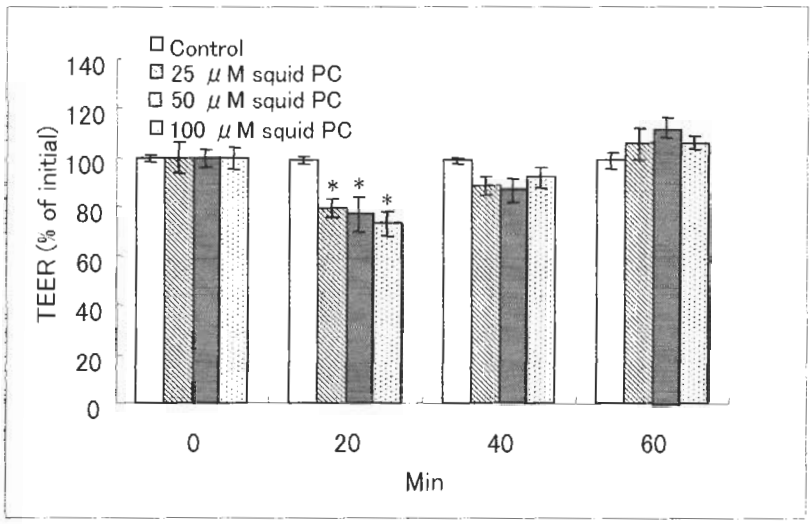


(A)



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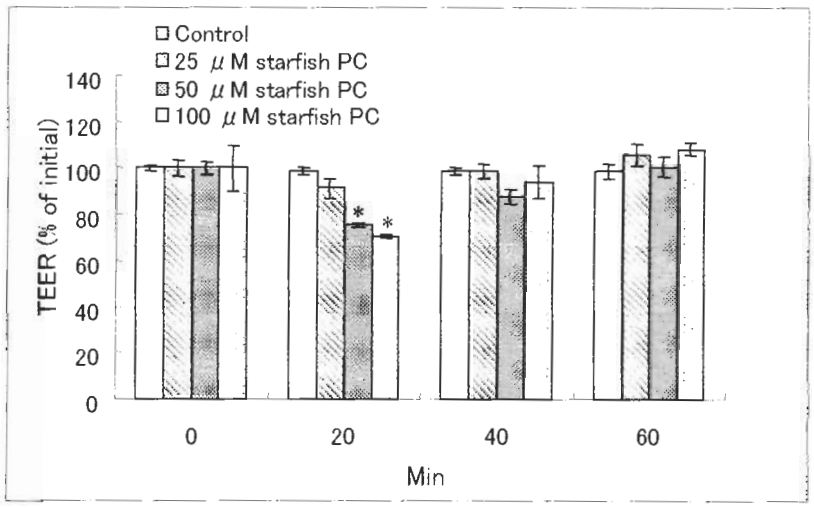


(C)

Fig. 2. Effect of soy PC (A), squid meal PC (B) and starfish PC (C) on TEER of Caco-2 cells grown on filter.

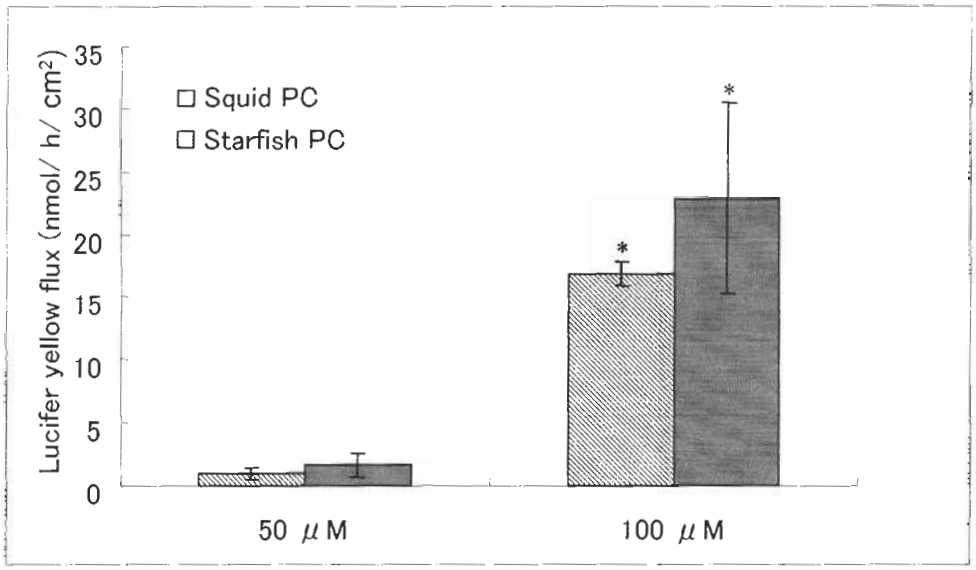
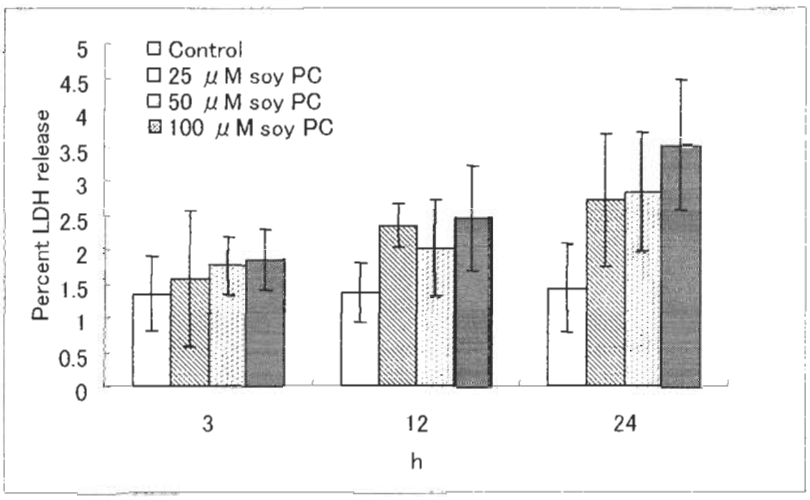
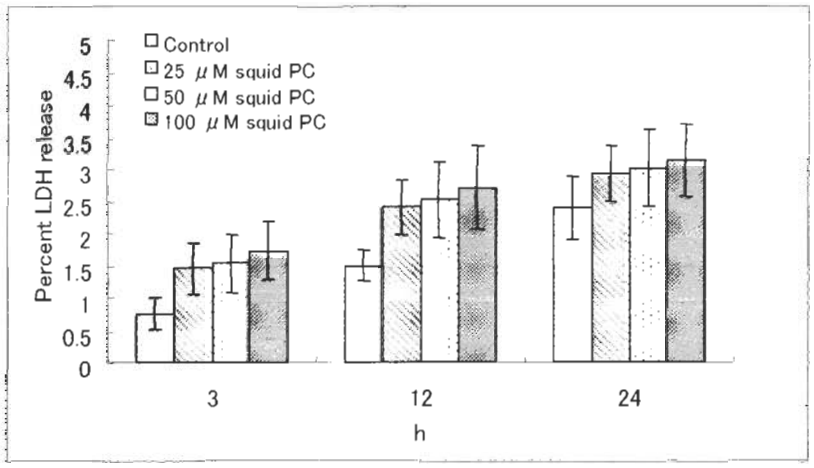


Fig. 3. Lucifer yellow flux of a cultured Caco-2 monolayer.



(A)



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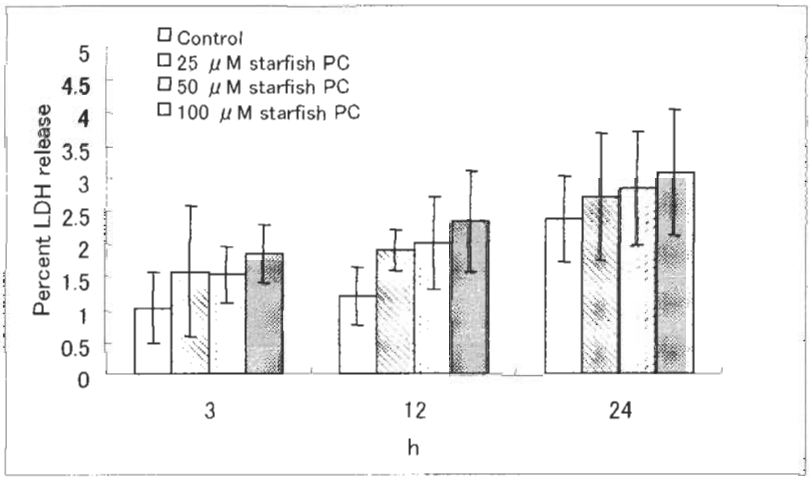

(C)

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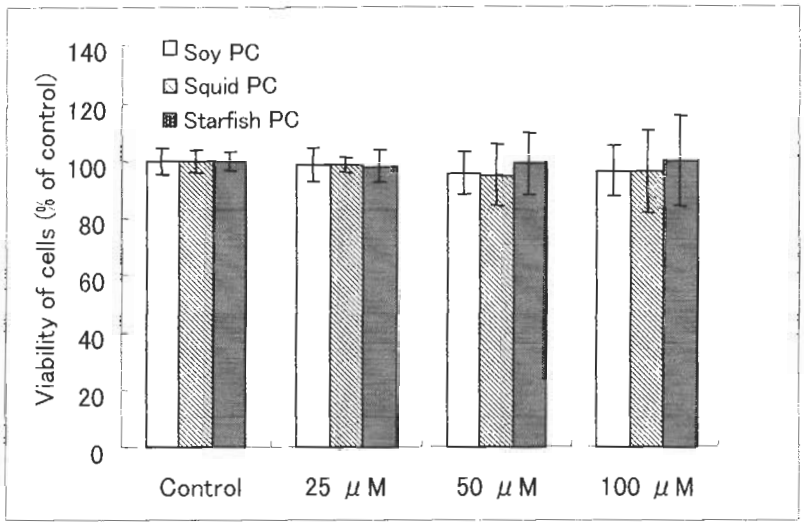

Fig. 5. Cell viability after 24 h incubation with different concentrations of soy, squid meal and starfish PC.

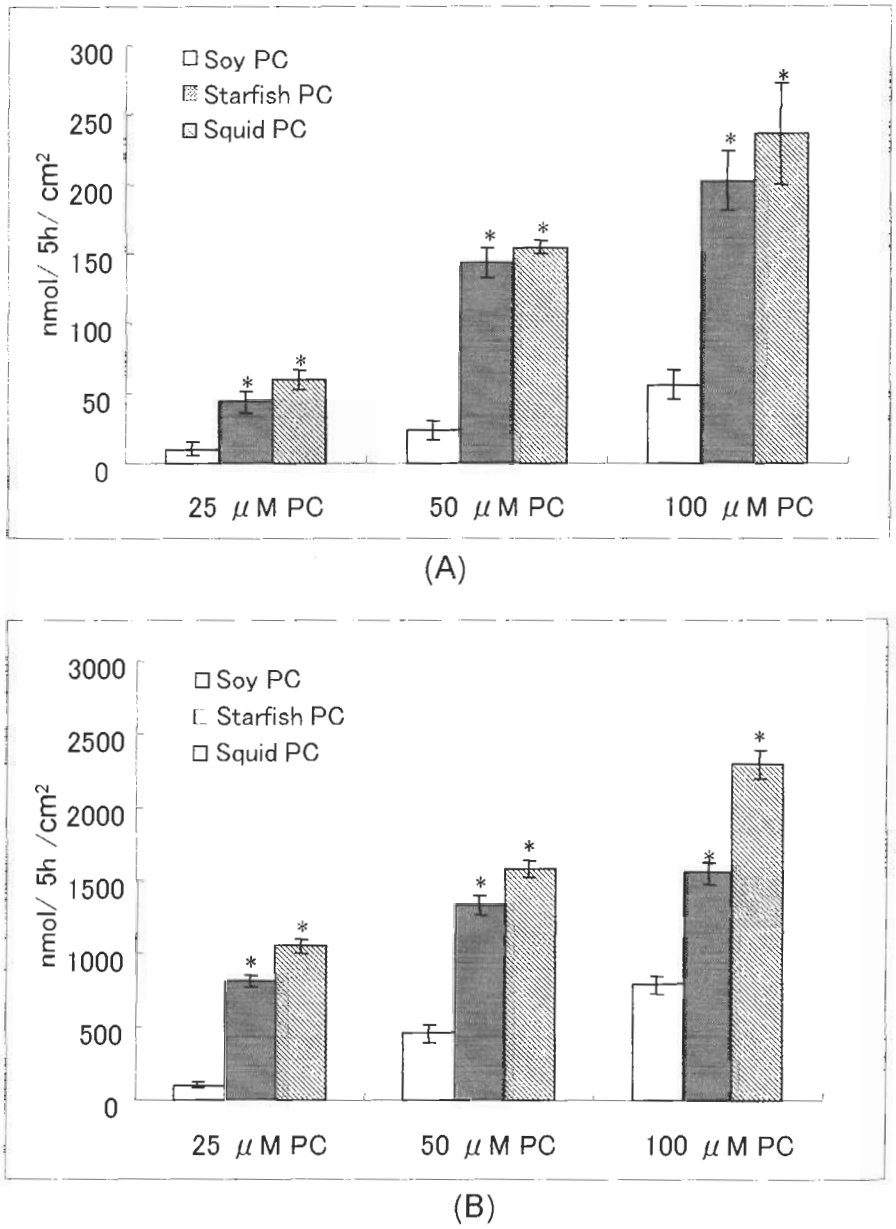

Fig. 6. Dose-dependent transport (A) and uptake (B) of PC liposomes across the Caco-2 monolayer.

Table 1

Fatty acid composition of soy, squid meal and starfish phosphatidylcholine

Fatty acid	%		
	Soy PC	Squid meal PC	Starfish PC
C 16:0 (Palmitic acid)	12.5	35.2	3.4
C 18:0 (Stearic acid)	0.2	1.3	9.5
C 18:1 (Oleic acid)	14.8	2.4	6.7
C 18:2 (Linoleic acid)	63.4	1.0	--
C 20:1 (Eicosenoic acid)	--	0.4	11.4
C 20:4 (Arachidonic acid)	--	1.0	5.6
C 20:5 (EPA)	--	9.2	47.3
C 22:5 (Docosapentaenoic acid)	--	--	1.5
C 22:6 (DHA)	--	42.6	8.3