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Lymphatic absorption of plasmalogen in rats

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Plasmalogen is a subclass of phospholipids that is widely distributed in man and animals. Many physiological roles have been proposed for this lipid; however, there have been no reports on the intestinal absorption of plasmalogen. In the present study, we examined lymphatic absorption of plasmalogen after the duodenal infusion of emulsified brain phospholipids (BPL) containing plasmalogen (22 mol % of total phospholipids) and soyabean lecithin (SPL) (100 g emulsified phospholipid/l). Male Wistar rats with implanted cannulas in the mesenteric lymph duct and the duodenum were kept in a Bollman-type restraining cage, and were infused the emulsion after 1 d recovery with duodenal infusion of a glucose–NaCl solution. Lymphatic plasmalogen output was increased at 2–4 h after the switch to BPL emulsion, and peaked at 4–6 h. However, no increases were observed after SPL infusion. Lymphatic recovery of plasmalogen for 8 h was 198 nmol, which was 0·22 mol % of the total plasmalogen disappeared from the intestine. We did not detect any increases in long-chain fatty aldehydes, which are the degradation product of plasmalogen, either in the blood or the small intestine. We conclude that a small percentage but a significant amount of the plasmalogen was absorbed into the lymph.

Plasmalogen: Phospholipids: Lymphatic absorption: Rats

Plasmalogen has a vinyl-ether double bond in the *sn*-1 position (1-O-alk-1′-enyl) that is susceptible to oxidative molecules (Zommara *et al.* 1995; Zoeller *et al.* 1999). While the role of plasmalogen is not yet fully understood, a protective role against oxidative stress has been proposed in cerebral and cardiac tissues and plasma lipoproteins (Zoeller *et al.* 1988; Engelmann *et al.* 1994; Jurgens *et al.* 1995). These previous studies suggest that increases in plasmalogen levels in plasma lipoprotein protect against cholesterol oxidation and lower the incidence of CHD. Plasmalogen may be an endogenous lipidaemic antioxidant. Many reports also suggest that plasmalogen has a role in the maintenance of cell membrane dynamics as a structural component (Glaser & Gross, 1994). Recently, Farooqui *et al.* (1997) reported that plasmalogen deficiency is involved in nerve degeneration in Alzheimer's disease.

Plasmalogen is widely distributed in human and animal tissues. The brain, heart, lung and erythrocytes contain relatively high levels of ethanolamine plasmalogens (Nagan & Zoeller, 2001), and many foods also contain plasmalogen (Blank *et al.* 1992). Phospholipids in the diet are absorbed via the lymph; however, there are no reports on the intestinal absorption of ingested plasmalogen or on the fate of the vinyl-ether double bond in plasmalogen after ingestion. The aims of the present study were to examine the lymphatic absorption of plasmalogen extracted from the brain as glycerophospholipids, and to examine its degradation into fatty aldehydes during intestinal absorption.

Methods

Animals and diets

Male Wistar rats (Japan SLC, Hamamatsu, Japan), weighing about 270 g, were given free access to tap water and a semi-purified casein–sucrose-based stock diet (American Institute of Nutrition, 1977, 1980) for a 6 d acclimatization. A polyvinyl tube (SV-35, internal diameter 0·5 mm, external diameter 0·9 mm; Natsume Seisakusyo, Tokyo, Japan) and a silastic tube (Silascon No. 00, internal diameter 0·5 mm, external diameter 1·0 mm; Dow Corning Co., Kanagawa, Japan) were implanted into the main mesenteric lymph duct (Bollman *et al.* 1948) and the duodenum, respectively, of the acclimatized rats under anaesthesia (pentobarbital sodium, 30 mg/kg body weight; Abbott, North Chicago, IL, USA). The cannulated rats were kept in Bollman-type restraining cages with a continuous infusion (3 ml/h) of a solution containing L-glucose (135 mmol/l) and NaCl (85 mmol/l). After a 1 d recovery period, the lymph fluid was collected for 1 h (the initial value, 0 time); the infusate was then switched to the test lipids (100 g phospholipid/l) emulsified by 10 g sodium taurocholate/l for 1 h (administration period) before being

Abbreviations: BPL, brain phospholipids; SPL, soyabean phospholipids.

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switched again to the glucose–NaCl solution. All experiments were done in a room with controlled temperature (22–24°C), relative humidity (40–60 %) and lighting (lights on 08.00–20.00 hours). The mesenteric lymph fluid was collected every 2 h for 8 h after the switch to the test lipid emulsion. Aortic blood was collected with a heparinized syringe 8 h after the switch to the test emulsion under a pentobarbital anaesthesia, and the rats were killed. Blood was separated into plasma and blood cells by centrifugation. The blood cells were washed twice with saline (9 g NaCl/l). The small intestine, caecum and the colon were immediately removed with their contents after ligation of both ends of each segment. These were frozen using liquid N_2, and stored at −80°C until subsequent analyses.

The present study was approved by the Hokkaido University Animal Committee, and animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

Test lipids used in experiments were a purified soyabean phosphatidylcholine (SPL, 980 g soyabean lecithin/kg, EPIKURON200; Lucus Meyer GmbH, Hamburg, Germany) and brain phospholipids (BPL) containing more than 200 g plasmalogen/kg. Brain lipid was extracted from the bovine brain (brain glycerophospholipids) by the method of Folch (1942) with some modifications. The plasmalogen and total phospholipid contents in the extracted BPL fractions were 224 and 821 g/kg, respectively.

Analytical methods

Total lipids in the lymphatic fluid, blood plasma and cells, and gastrointestinal contents were extracted using chloroform–methanol–saline (10:5:3, by vol.) (Folch *et al.* 1957). Plasmalogen was measured by the I addition method (Gottfried & Rapport, 1962; Williams *et al.* 1962; Huque *et al.* 1987), in which the vinyl-ether double bonds in plasmalogen specifically react to I in the presence of methanol. I consumption by the vinyl-ether moiety was measured photometrically at 355 nm. Plasmalogen concentrations evaluated by the I addition method were closely correlated with the values obtained by the *p*-nitrophenylhydrazone method (Pries & Böttcher, 1965; *r* 0·984, data not shown), in which plasmalogen was degraded to fatty aldehyde with sulfuric acid (0·3 mol/l). Fatty aldehydes in the blood plasma and small intestine were measured using the *p*-nitrophenylhydrazone method with sulfuric acid (5 mmol/l). The specificity of the I addition method for the vinyl-ether moiety in plasmalogen was very high; that is, I consumption by soyabean lecithin and cholesterol was just 0·14 and 0·07 mol %, respectively, of that by plasmalogen.

Phospholipid content in the extracted lipid fraction was measured by phosphate (Phospholipid-test Wako; Wako Pure Chemical Industries, Osaka, Japan), and triacylglycerol content was assayed by an enzymic procedure (Triglyceride G-test Wako; Wako Pure Chemical Industries).

Calculations and statistical analysis

We calculated the molar amounts of plasmalogen and phospholipids using 777·3, 770·1 and 729·5 as the average molecular weights of phospholipids in SPL, BPL and plasmalogen in BPL, respectively.

Plasmalogen absorption rate shown in Table 1 was estimated from the disappearance rate of the lipids from

Table 1. Plasmalogen and phospholipid contents in each part of the alimentary tract and plasmalogen levels of blood 8 h after the infusion of a lipid emulsion containing soyabean phospholipids (SPL) or brain phospholipids (BPL)

(Mean values and standard errors of the mean)

	Infused phospholipids	Plasmalogen		Phospholipids	
		Mean	SEM	Mean	SEM
Small intestine (μmol)†	SPL	1·18	0·22	40·6	5·3
	BPL	3·65*	0·77	39·9	3·6
Caecum (μmol)	SPL	2·30	0·30	50·7	5·6
	BPL	35·3**	4·5	160**	28·5
Colon (μmol)	SPL	0·39	0·16	4·2	1·4
	BPL	4·10	2·57	17·6	11·8
Total (μmol)‡	SPL	3·90	0·34	95·5	9·07
	BPL	42·9**	1·97	217**	18·1
Absorption rate (%)§	BPL	53·4	2·10		
Blood plasma (nmol/l)	SPL	25·4	2·8		
	BPL	42·5‖	10·5		
Blood cells (nmol/l)	SPL	460	103		
	BPL	614	106		

Mean values for the SPL and BPL groups were significantly different by the Mann–Whitney test (* $P<0·05$; ** $P<0·01$, n 5).

† Values for the small intestine, but not for the caecum and colon, contain mucosal lipids.

‡ Total values are the sum of the amounts of each lipid in the three intestinal segments.

§ Absorption rate of plasmalogen in BPL was estimated as disappearance rate of the vinyl-ether double bonds from the intestines. Details are shown on p. 29.

‖ There was a tendency for higher values in the BPL group than in the SPL group by the Mann–Whitney test ($P=0·076$).

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the alimentary tract using the following equation:

$$\text{Absorptive rate}\,(\%) = (\text{ingested plasmalogen}\,(\mu\text{mol}) - \text{remaining plasmalogen}\,(\mu\text{mol}))/(\text{ingested plasmalogen}\,(\mu\text{mol})) \times 100.$$

The value of remaining plasmalogen was evaluated by subtracting the mean value of total content (the sum of three intestinal segments) in the SPL group from the total value of each rat in the BPL group.

The results were analysed by two-way ANOVA (Fig. 1). Duncan's multiple-range test was used to determine whether the mean values were significantly different ($P<0·05$) (Duncan, 1995). The Mann–Whitney test was used in Table 1. These statistical analyses were done by SAS (SAS version 6.07; SAS Institute Inc., Cary, NC, USA).

Results

Final body weight in groups infused with SPL and BPL were 306 (SEM 5·0) and 306 (SEM 6·0) g, respectively.

Plasmalogen output in the mesenteric lymph was increased 2 h or later after the switch to BPL emulsion, and reached a peak value at 4–6 h after the start of BPL infusion (Fig. 1 (a)). Plasmalogen output was not increased after SPL infusion. Total output of plasmalogen for 8 h (net increase) was 198 (SEM 51·4) nmol/8 h, which is just 0·215 mol % of the total infused plasmalogen. The amount of plasmalogen infused was 92 μmol, and total phospholipids were 320 μmol in the BPL group and 378 μmol in the SPL group. Lymphatic output of total phospholipids was immediately increased after the infusion of SPL, but not after the infusion of BPL (Fig. 1 (b)). Output reached a peak value at 2–4 h after the switch in the SPL group, and at 4–6 h in the BPL group. The net phospholipids increase in the SPL group was more than 3-fold higher than that in the BPL group.

The plasmalogen and phospholipid levels in the caecal contents were much higher in the BPL group than in the SPL groups (Table 1). Plasmalogen absorption rate (disappearance rate) estimated from the lipids remaining in the whole alimentary tract was approximately 50 mol % 8 h after the administration of BPL.

Plasmalogen concentration in the blood plasma 8 h after the infusion of test emulsion was 67 % higher in the BPL group than in the SPL group ($P<0·076$; Table 1). There were no differences in long-chain fatty aldehyde levels in both the blood plasma and the small intestine with their luminal contents between the SPL and BPL groups (data not shown).

Discussion

The present study showed that enterally infused plasmalogen was released into the mesenteric lymph, and change in the lymphatic plasmalogen profile was similar to that of total phospholipid output after an infusion of BPL. These results suggest that lipid-bearing vinyl-ether double bonds are absorbed as a phospholipid. The net increase in plasmalogen output in the lymph was approximately 0·2 μmol for 8 h, which is much lower than the total amount of infused plasmalogen (92 μmol, recovery rate 0·22 %). In contrast, the disappearance rate of plasmalogen from the whole gut was 54·3 %, which is much higher than the lymphatic recovery rate.

Lymphatic recovery rate of the acyl groups in the infused SPL was 74·7 % (infused acyl groups in phosphatidylcholine, 378 μmol × 2; net increases in acyl groups in phospholipids, 46·9 μmol × 2 (Fig. 1 (b)); in triacylglycerols, 157 μmol × 3 (Fig. 1 (c))). The lymphatic triacylglycerol output may be derived from the acyl groups (fatty acids) of soyabean phosphatidylcholine. The reason for the low recovery of the lymph plasmalogen is not the low collection rate of the mesenteric fluid, but the degradation of plasmalogen in the small intestine.

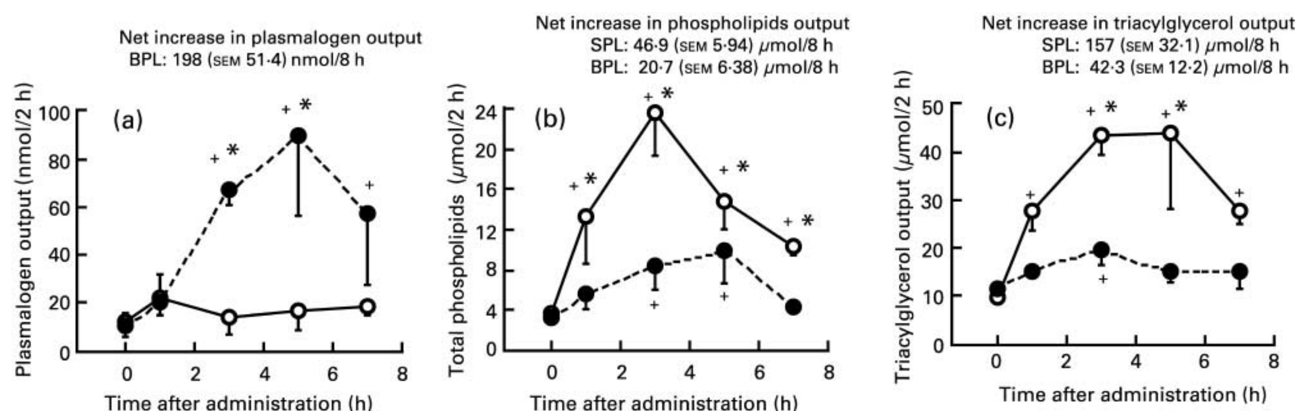

Fig. 1. Lymphatic output of plasmalogen (vinyl-ether double bonds; (a), total phospholipids (b) and triacylglycerols (c) after a duodenal infusion of soyabean phosphatidylcholine (SPL; 378 μmol) (-○-) or brain phospholipids (BPL) (-●-) in mesenteric lymph-cannulated rats. Mean values are shown, with standard errors of the mean represented by vertical bars (*n* 5). * Mean values were significantly different between the SPL and BPL groups at the same time. Significant increases against the values at time 0 in each group are shown by + ($P<0·05$). Each point represents collection of the lymph for a 2 h period; for example, the point at 1 h shows the values for the 0–2 h collection. Values at time 0 represent the 2 h collection before the infusion of the test lipid emulsion. The net increases in output above the graphs show the total lymph output subtracted from the basal (time 0) values for 8 h after the start of a test emulsion. *P* values for (a) estimated by two-way ANOVA were $P=0·002$ for Lipid (P), $P=0·072$ for Time (T), $P=0·073$ for P×T; for (b) they were $P<0·001$ for Lipid (P), $P<0·001$ for Time (T), $P=0·002$ for P×T; for (c) they were $P<0·001$ for Lipid (P), $P=0·007$ for Time (T), $P=0·098$ for P×T.

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Plasmalogens are degraded into fatty aldehydes at the *sn*-1 vinyl-ether double bond by lysoplasmalogenase (*EC* 3.3.2.2) (Jurkowitz-Alexander *et al.* 1989; Jurkowitz *et al.* 1999). Oxygen radicals also degrade the vinyl-ether moiety and produce long-chain fatty aldehydes (Stadelmann-Ingrand *et al.* 2001). However, any increase in the long-chain fatty aldehydes in the lymph, blood plasma and the intestinal tissues was not observed (data not shown), which suggests that the very low recovery of lymphatic plasmalogen was not associated with increases in the cleavage products of vinyl-ether bonds. There is a possibility that saturation of the vinyl-ether double bonds occurs in the intestine. Further study is therefore needed to clarify this activity.

We observed a tendency towards an increase in blood plasmalogen levels. Although the main mesenteric lymph duct was obstructed to collect the lymph fluid, there are a few minor mesenteric lymph ducts. The finding that BPL infusion increased the blood level of plasmalogen by an average of 70 % is important because plasmalogen in blood plasma has been proposed as a lipidaemic antioxidant of lipoproteins (Jurgens *et al.* 1995; Hahnel *et al.* 1999). The increase in blood plasmalogen is expected to be much more without obstruction of the main mesenteric lymph duct because about 200 nmol plasmalogen was absorbed, which was much higher than the plasma level before administration. The rate of plasmalogen release into the lymph was very low; however, it may have been sufficient to increase the plasmalogen level in the blood plasma, and for the prevention of CHD or other illness related to oxidative stresses.

We have previously shown that most plasmalogen included in the diet was saved from gastric acid degradation (H Hara and T Wakisata, unpublished results). The present study reveals that dietary plasmalogen is absorbed and released into the lymph as a phospholipid.

References

American Institute of Nutrition (1977) Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. *J Nutr* **107**, 1340–1348.

American Institute of Nutrition (1980) Second report of the ad hoc Committee on Standards for Nutritional Studies. *J Nutr* **110**, 1726.

Blank ML, Cress EA, Smith ZL & Snyder F (1992) Meats and fish consumed in the American diet contain substantial amounts of ether-linked phospholipids. *J Nutr* **122**, 1656–1661.

Bollman JL, Cain JC & Grindlay JH (1948) Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. *J Lab Clin Med* **33**, 1349–1352.

Duncan DB (1995) Multiple range and multiple F tests. *Biometrics* **11**, 1–42.

Engelmann B, Brautigam C & Thiery J (1994) Plasmalogen phospholipids as potential protectors against lipid peroxidation of low density lipoproteins. *Biochem Biophys Res Commun* **204**, 1235–1242.

Farooqui AA, Rapoport SI & Horrocks LA (1997) Membrane phospholipid alterations in Alzheimer's disease: deficiency of ethanolamine plasmalogens. *Neurochem Res* **22**, 523–527.

Folch J (1942) Brain cephalin, a mixture of phosphatides. Separation from it of phosphatdylserine, phosphatidylethanolamine, and a fraction containing an inositol phosphatides. *J Biol Chem* **146**, 35–44.

Folch J, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.

Glaser PE & Gross RW (1994) Plasmenylethanolamine facilitates rapid membrane fusion: a stopped-flow kinetic investigation correlating the propensity of a major plasma membrane constituent to adopt an HII phase with its ability to promote membrane fusion. *Biochemistry* **33**, 5805–5812.

Gottfried EL & Rapport MM (1962) The biochemistry of plasmalogens. I. Isolation and characterization of phosphatidal choline, a pure native plasmalogen. *J Biol Chem* **237**, 329–333.

Hahnel D, Beyer K & Engelmann B (1999) Inhibition of peroxyl radical-mediated lipid oxidation by plasmalogen phospholipids and alpha-tocopherol. *Free Radic Biol Med* **27**, 1087–1094.

Huque T, Brand JG, Rabinowitz JL & Gavarron FF (1987) A comparative study of three methods for the estimation of total plasmalogens in lingual taste epithelium and other tissues. *Comp Biochem Physio B* **86**B, 135–139.

Jurgens G, Fell A, Ledinski G, Chen Q & Paltauf F (1995) Delay of copper-catalyzed oxidation of low density lipoprotein by in vitro enrichment with choline or ethanolamine plasmalogens. *Chem Phys Lipids* **77**, 25–31.

Jurkowitz MS, Horrocks LA & Litsky ML (1999) Identification and characterization of alkenyl hydrolase (lysoplasmalogenase) in microsomes and identification of a plasmalogen-active phospholipase A2 in cytosol of small intestinal epithelium. *Biochim Biophys Acta* **1437**, 142–156.

Jurkowitz-Alexander M, Ebata H, Mills JS, Murphy EJ & Horrocks LA (1989) Solubilization, purification and characterization of lysoplasmalogen alkenylhydrolase (lysoplasmalogenase) from rat liver microsomes. *Biochim Biophys Acta* **1002**, 203–212.

Nagan N & Zoeller RA (2001) Plasmalogens: biosynthesis and functions. *Prog Lipid Res* **40**, 199–229.

Pries C & Böttcher CJF (1965) The determination of free and plasmalogen-bound aldehydes in lipid fraction. *Biochim Biophys Acta* **98**, 329–334.

Stadelmann-Ingrand S, Favreliere S, Fauconneau B, Mauco G & Tallineau C (2001) Plasmalogen degradation by oxidative stress: Production and disappearance of specific fatty aldehydes and fatty alpha-hydroxyaldehydes. *Free Radic Biol Med* **31**, 1263–1271.

Williams JNJ, Anderson CE & Jasik AD (1962) A sensitive and specific method for plasmalogens and other enol esters. *J Lipid Res* **3**, 378–381.

Zoeller RA, Lake AC, Nagan N, Gaposchkin DP, Legner MA & Lieberthal W (1999) Plasmalogens as endogenous antioxidants: somatic cell mutants reveal the importance of the vinyl-ether. *Biochem J* **338**, 769–776.

Zoeller RA, Morand OH & Raetz CR (1988) A possible role for plasmalogens in protecting animal cells against photosensitized killing. *J Biol Chem* **263**, 11590–11596.

Zommara M, Tachibana N, Mitsui K, *et al.* (1995) Inhibitory effect of ethanolamine plasmalogen on iron- and copper-dependent lipid peroxidation. *Free Radic Biol Med* **18**, 599–602.