CHAPTER 3

Marine By-product Phospholipids as Booster of Medicinal Compounds

Koretaro Takahashi[*,1] and **Yoshikazu Inoue**[†]

Contents

Abstract

Marine phospholipids are defined as phospholipids containing docosahexaenoic acid (DHA) or eicosapentaenoic acid that would be more effective than fish oil, which is mostly composed of triacylglycerol, in exerting health benefits. Marine phospholipids would boost the effect of both the health-beneficial hydrophilic and the hydrophobic compounds such as cell differentiators, anticancer compounds, and antiobesity compounds. When marine phospholipids are served as liposomal drinks, they would be more effective than adding into solid foods or feeds. As long as the liposome bilayer is basically composed of marine phospholipids, they would promote the encapsulated functional compounds. And this is the principal advantage of choosing marine phospholipids as liposomal membrane. Bioconversion of marine phospholipid would also be advantageous in delivering DHA into the desired tissue.

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For example, lysophosphatidylserine obtained through phospholipase D-mediated transphosphatidylation and phospholipase A_1 or *sn*-1 positional specific lipase-mediated partial hydrolysis seemed to be the most effective chemical form in delivering DHA into brain.

I. INTRODUCTION

Marine (fisheries) by-products are rich in docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA). Although the most abundant DHA- or EPA-rich lipid class in most of the marine by-products is triacylglycerol (TG), some of the by-product sources are rich in DHA- or EPA-containing phospholipids. And these DHA- or EPA-containing phospholipids are often called marine phospholipids. Well-known sources for DHA-containing phospholipids are squid skin, squid muscle, squid connective tissues, and testis as well as ovary of fish and shellfish. On the other hand, recently known sources for EPA-bounded phospholipids are starfish (*Asterias amurensis*) internal organs and gonads. Typical sources for both DHA- and EPA-abundant phospholipids are those of giant scallop internal organs, salmon heads, and krills. We can say that marine phospholipids are rich in fisheries by-products or poorly used marine bioresources.

II. MARINE PHOSPHOLIPID MUST BE MORE EFFECTIVE THAN FISH OIL TG ON HEALTH BENEFITS

Health benefits of DHA or EPA have been widely recognized. However, DHA or EPA itself is just a moiety of lipid molecules such as TG or phospholipids. TG is a typical hydrophobic compound, while on the other hand, phospholipids are amphiphilic compounds. For this reason, phospholipid form DHA and EPA are considered to be much more bioavailable than those of TG forms. In fact, Galli *et al*. (1992) has borne out that phospholipid form polyunsaturated fatty acid dosing should result in a higher polyunsaturated fatty acid content in plasma than TG form polyunsaturated fatty acid dosing. It has been discussed in detail that marine phospholipids must be more efficient than marine TG (i.e., fish oil) in delivering $n-3$ polyunsaturated fatty acids to desired tissues (Lu *et al*., 2011). Fish oils are well known to decrease the levels of total cholesterol, blood TG content, and low density lipoprotein (LDL), while on the other hand increase high density lipoprotein (HDL) in blood. However, in practical cases, decreasing the TG content and increasing the HDL content in blood cannot be achieved by moderate intake of fish oil. Large amount of fish oil administration is necessary for this purpose

TABLE 3.1 Lipid content in the serum of rats fed with DHA- or EPA-containing triacylglycerol (TG) and phosphatidylcholine (PC)

	EPA-PC	EPA-TG	DHA-TG	Control
HDL cholesterol	64.9 ± 5.5^{a}	54.1 ± 10.1^{b}	53.6 ± 9.2^{b}	57.4 ± 6.0^{b}
LDL cholesterol	4.5 ± 0.4^{b}	5.2 ± 0.6^{a}	5.1 ± 0.6^{a}	5.1 ± 0.5^{a}
Total cholesterol	55.1 ± 3.2^{b}	61.9 ± 5.3^{a}	64.4 ± 6.1^{a}	66.0 ± 7.8^{a}
Free cholesterol	11.1 ± 1.7	13.0 ± 1.0	11.9 ± 3.0	12.6 ± 1.3
Cholesterol ester	43.9 ± 2.3^{b}	48.9 ± 5.9^{b}	52.4 ± 5.3^{a}	53.6 ± 7.3^{a}
Phospholipid	139.9 ± 18.1^{a}	102.4 ± 13.7^{c}	108.0 ± 16.3^{c}	124.7 ± 13.3^{b}

mg/dL serum.
Shina *et al.* (2008).
Different superscript letters are significant different ($P<0.05$).

compared to marine phospholipids. In fact as shown in Table 3.1, we recently observed that among marine lipids, for example, the phosphatidylcholine (PC) form EPA (EPA-PC), TG form DHA (DHA-TG) and EPA (EPA-TG), EPA-PC was the only lipid molecular species effective in increasing the level of HDL with moderate amount of intake. Krill oil contains approximately 35% phospholipids. The effect of krill oil on serum lipids and markers of oxidative stress and inflammation have recently been studied (Ulven *et al.*, 2011). Molecular species of krill phospholipids are also well studied (Winther *et al.*, 2011). It was observed that the metabolic effects of krill oil rich in marine phospholipids was comparable to fish oil even if the EPA+DHA dose in the krill oil was 63% of the fish oil. A typical health benefit of fish oil is the prevention of overproduction of arachidonic acid-derived eicosanoids which often increase the risk of cancer, thrombosis, allergy, and other diseases. In this sense, marine phospholipids would be more effective when they are served in same amount. Moreover, Di Marzo *et al.* (2010) showed that DHA in the brain increased significantly after krill oil administration, when compared to control animals. It is interesting to note that $n-3$ fatty acids linked to phospholipids may be differently distributed in the body compared to $n-3$ fatty acids in other lipid molecular forms. Wijendran *et al.* (2002) found that efficacy of dietary arachidonic acid provided as phospholipid as substrate for brain arachidonic acid accretion in baboon neonates is much higher than TG. And this also tells that phospholipid form must be more effective than TG form in reaching the desired polyunsaturated fatty acid to the desired tissue when administrated. There are many kinds of phospholipid classes. Recently, it was observed that among the DHA-containing phospholipids, as substrates for DHA accretion in fetus rat brain, lysophosphatidylserine (LPS) form DHA seemed to be the most effective DHA carrier. As depicted in Table 3.2, the percentage of DHA in fatty acid composition of the rat fetus brain fed with LPS-added diet was

TABLE 3.2 Fatty acid composition of the fetal brain lipid in pregnancy rat administrated phospholipid samples by probe (%)

Fatty acid	DHA-PS	DHA-LPS	DHA-PC	DHA-LPC	Control
C14:0	2.3±0.2	2.1±0.2	2.7±0.7	2.2±0.1	2.2±0.1
C16:0	38.7±0.9	38.2±0.7	38.0±1.5	38.4±0.6	38.8±0.4
C18:0	16.9±1.6	18.4±1.5	16.9±1.9	18.5±1.6	16.9±1.0
C18:1	18.4±1.6	16.2±1.8	19.0±2.5	16.7±1.9	17.7±1.0
C18:2	1.2±0.1	1.4±0.9	1.2±0.4	1.1±0.2	1.0±0.1
C20:4	12.9±0.4	12.2±0.4	12.5±0.7	13.0±0.5	12.9±0.2
C22:6	9.5±1.3	11.5±1.2[a]	9.8±1.1	10.1±1.2	9.5±0.8

Onoyama *et al.* (2011).
Datum with superscript letter is significant different ($P<0.05$).

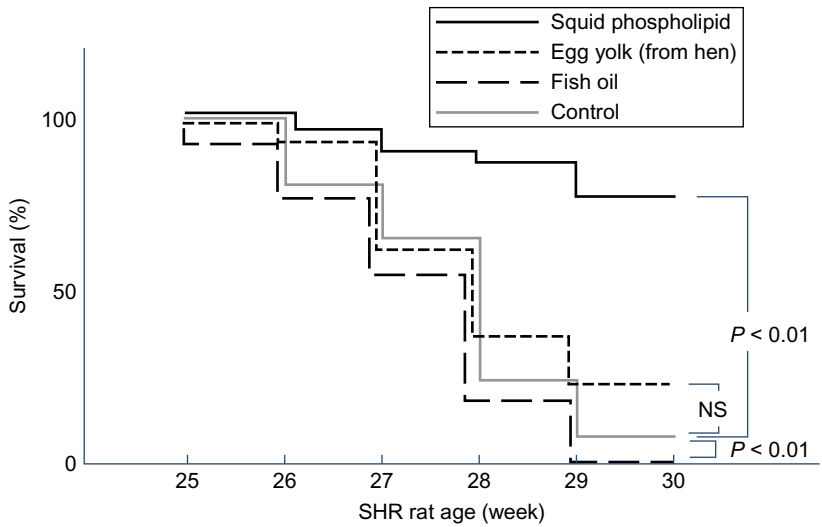

FIGURE 3.1 Survival of SHR rats (easily die by apoplexy) served with phospholipid form DHA (squid PC), fish oil, and egg yolk PC (Inoue, 2001).

the highest. This LPS form DHA can be obtained through bioconversions using phospholipase D to convert DHA-containing PC into phosphatidylserine (PS) and then using phospholipase A_1 or the position *sn*-1 specific lipase to convert PS into LPS.

A typical feature that tells the importance of the backbone of phospholipid molecule which DHA bounds is the prevention effect on apoplexy. A combination of phosphoryl base group with DHA must be crucial to exert antiapoplexy function as shown in Fig. 3.1. Among fish oil TG, egg yolk phospholipid, and squid phospholipid which is rich in *sn*-2

DHA-bounded phospholipids, the squid phospholipid is the only functionally available lipid chemical form in preventing apoplexy (Inoue, 2001).

III. BOOSTING EFFECT ON CANCER CELL DIFFERENTIATION

Applying marine phospholipids rich in DHA and EPA must also be beneficial for the treatment of cancer. Because DHA-containing phospholipids have been recognized as lipids that promote cancer cell differentiation induced by some cell differentiators. When cancer cell differentiates, it becomes mortal. And that should help the therapy of cancer. Tochisawa (1997a) showed that DHA-containing phospholipids promote cancer cell differentiation induced by retinoic acid (Fig. 3.2), and Hosokawa *et al.* (2001) showed that DHA-containing phospholipids promote cancer cell differentiation induced by dibutyryl cyclic adenosine monophosphate on nonfixative cancer cells such as leukemia HL-60 cell line. On the other hand, Hossain *et al.* (2006) showed that DHA-containing phospholipids promote cancer cell differentiation induced by butylate for fixative cancer cells such as colon cancer. However, on fixative cancer cells such as colon cancer, PS with the same fatty acid combination exerted more effectively than PC on the promotional effect. Hossain *et al.* (2006) also showed that the suppression of colorectal cancer (Caco-2) cells by the marine phospholipids may not only caused by the promotional effect of cancer cell differentiation but also by the induction of apoptosis with other bioactive compounds as borne out through enrichment factor comparison experiments which is an index of

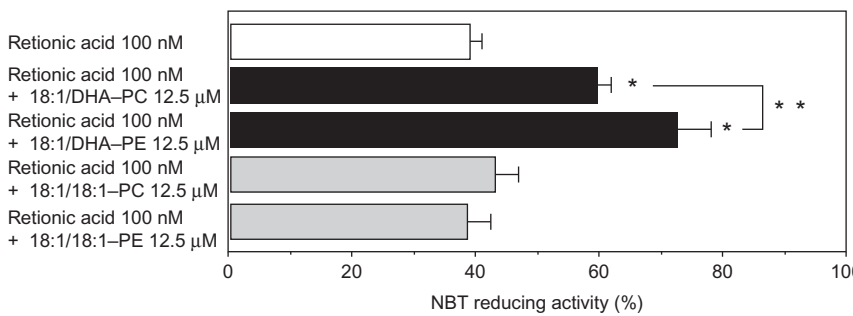

FIGURE 3.2 Effect of retinoic acid (RA) and various lipids on cell differentiation of HL-60 cells (Tochisawa *et al.*, 1997). HL-60 cells (5×10^4) were incubated with the individual lipids for 24h, then RA was added followed by incubation for additional 72h. Nitroblue tetrazolium (NBT) reducing activity in HL-60 cells was assayed. Data shown as mean value±SD. *$p<0.01$ versus control. **$p<0.01$ versus RA.

DNA fragmentation relative to control. The enrichment factor was significantly high in squid PC and much higher in squid PC-bioconverted PS when combined with sodium butylate. Unfortunately, PS is poorly found in natural sources except brain and nerve tissues. For this reason, transphosphatidylation of marine PC to PS should become critical when obtaining DHA- or EPA-bounded PS in a reasonable price. The promotional effects on cell differentiation compounds must be very useful in cancer therapy, because DHA-containing phospholipids, the marine phospholipids, should decrease side effects of the anticancer drugs that seriously impair quality of life. For example, large amount of retinoic acid is usually prescribed as an anticancer drug for the lives of patients, and with too much retinoic acid, side effect seriously impairs the quality of life of the patients. Burns and Spector (1987) showed that uptake of Adriamycin was greater in DHA-enriched L1210 cells compared to other unsaturated fatty acid-enriched cells. This was also true for mitoxantrone (Burns *et al.*, 1988). In those experiments, free fatty acid form DHA was used which is no doubt less bioavailable than the phospholipid forms. There have been several reports that explain at least in part the mechanisms of how DHA-containing phospholipids exert promotional effects, as seen above, on functional compounds. Stillwell *et al.* (1993) considered that increase in permeability of the organelle biomembranes by the fusion of DHA-bounded phospholipid should be the reason why the cells become susceptible to medicinal substances. Tochisawa (1997) carried out a TNBS-quenching method by using DHA-PE and considered that easy incorporation of that phospholipid into the cell membrane and then increase in fluidity of the cell membrane owing to the property of DHA might be one reason why the HL-60 cells become susceptible to retinoic acid. They also observed that simultaneous addition of marine phospholipids with retinoic acid to the cell lines is not effective in promoting the cell differentiation unlike the preincubation with marine phospholipids and considered that DHA-containing phospholipids should be incorporated into the cells in advance to the incorporation of retinoic acid. On the other hand, Larsson *et al.* (2004) considered that DHA may also influence on gene expression, transcription factor activity, and signal transduction pathways. For example, 1-oleoyl-2-DHA-containing PE was shown to facilitate upregulating expressions of c-*jun* mRNA and c-Jun protein, while it was effective in downregulating c-*myc* mRNA (Hosokawa, 2004; Fig. 3.3). c-Jun protein is in part responsible for cell differentiation process, and c-Myc protein is responsible for cell cycle progression. Therefore, it is considered that phospholipids containing DHA may at least in part suppress leukemia by modulating the oncogene expressions. Moreover, Tochisawa (1997) got the evidence that protein kinase C is involved in the promotional effect on cell differentiation. He showed that the promotional effect was obviously impaired by protein kinase C inhibitor. He also showed that when HL-60 cell line is preincubated

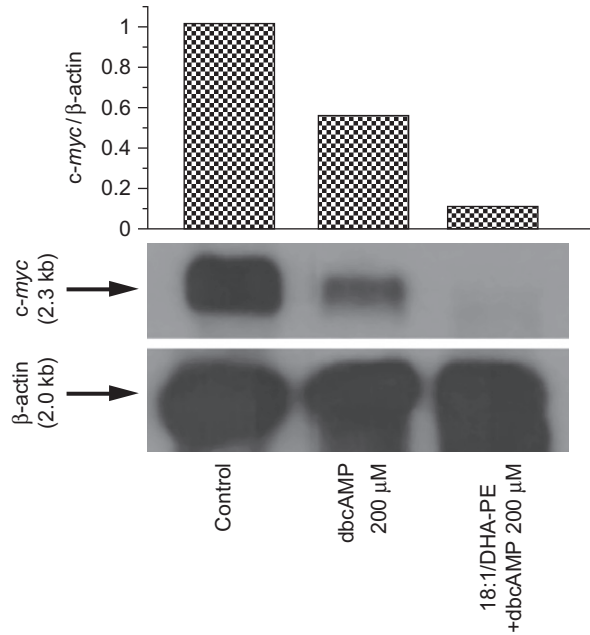

FIGURE 3.3 Downregulation of *c-myc* mRNA in HL-60 cells treated with dibutyryl cAMP (dbcAMP) and PE form DHA (Hosokawa, 2004). HL-60 cells (5×10^4) were incubated with (dbcAMP) after preincubation with 50 μM 1-oleoyl, 2-docosahexaenoyl-phosphatidyl-ethanolamine for 24 h. Nitroblue tetrazolium (NBT) reducing activity in HL-60 cells was assayed. Data shown as mean value±SD. *$p<0.01$ versus control. **$p<0.01$ versus RA.

with marine phospholipid prior to the addition of retinoic acid, the expression of mRNA of retinoic acid receptor α increases, which suggests that marine phospholipids should go into nucleus, and then enhances the susceptibility to retinoic acid. Konishi (2003) showed that DHA-containing PS may promote Caco-2 cell differentiation induced by sodium butylate via upregulation of tumor growth factor β-1 as shown in Table 3.3. On the other hand, polyunsaturated fatty acids are known to regulate peroxisome proliferators-activated receptor (PPAR) which is involved in cell differentiation, cell proliferation, and inflammatory responses (Grimaldi, 2001; Vamecq and Latruffe, 1999). DHA was found to induce apoptosis in vascular smooth muscle cells by activation of PPAR-α and p38 mitogen-activated protein kinases (Diep *et al.*, 2000).

TABLE 3.3 TGF β-1 expression of Caco-2 cells treated with DHA-PS and sodium butyrate

	TGF β-1 (ng/mg protein)
Control	213±9
DHA-PS	210±4
Sodium butylate (0.5mM)	269±8*
DHA-PS+sodium butylate	350±13*,**

Data are shown as means±SD (n=5). *p<0.01 versus control, **p<0.01 versus sodium butylate.
PS, phosphatidylserine; TGF, tumor growth factor.

IV. BOOSTING EFFECT ON CANCER SUPPRESSION

Marine phospholipids in the form of liposomes were borne out to be also useful in boosting the effect of water-soluble anticancer compounds such as β-glucan. Murakawa *et al*. (2007) carried out an *in vivo* study on mice to see the promotional effect on cancer tumor suppression when *Agaricus blazei* Murrill water extract (rich in β-glucan) encapsulated marine phospholipid is orally taken as liposomal drink. In their study, myeloma sp2 cells were first implanted (3.0×10^5 cells/BALB/c male mouse). After 20 days, mice with comparable tumor sizes were selected and divided into four groups: (i) control (only water served), (ii) 0.4mg/mL squid phospholipid liposome (PC/PS/cholesterol(Chol)=9/1/10 molar ratio) alone served as drink, (iii) 0.5mg/mL *A. blazei* Murrill water extract alone served as drink, and (iv) 0.4mg/mL squid phospholipid liposome (PC/PS/Chol=9/1/10 molar ratio) with 0.5mg/mL *A. blazei* Murrill water extract partially encapsulated served as drink. These drinks were orally administrated for 3weeks. The administrated drink amount per mouse was approximately 5mL per day. The results showed that by serving the mice with only low concentration of squid phospholipid liposome or *A. blazei* Murrill water extract individually, no appreciable suppression against cancer growth was observed. However, when both the squid phospholipid and *A. blazei* Murrill water extract were combined, suppression became noticeable as shown in Fig. 3.4. To see the reproducibility of this promotional effect on mushroom immune activator, they did another *in vivo* study with some modifications. Half number of myeloma sp2 cells (1.5×10^5 cells/BALB/c male mouse) compared to the prior experiment was implanted. Then after 20days, mice with comparable size were selected and divided into the four groups that are (i) control (only water served), (ii) 1.0mg/mL squid phospholipid liposome (PC/PS/Chol=9/1/10 molar ratio) alone served as drink, (iii) 1.0 mg/mL *A. blazei* Murrill water extract alone served as drink, (iv) 1.0mg/mL squid phospholipid liposome (PC/PS/Chol=9/1/10 molar ratio)

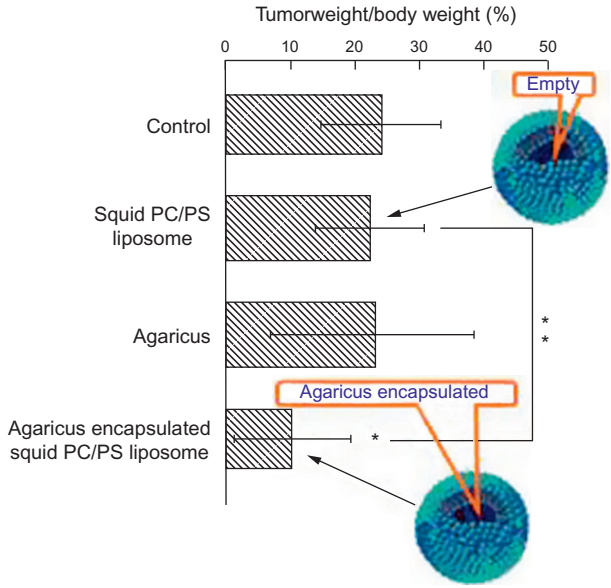

FIGURE 3.4 Tumor weight (%) against body weight of myeloma sp2-bearing BALB/c mice. Samples were administered 7 days after myeloma sp2 tumor (3.0×10^5 cells) implantation. Marine phospholipid based liposomes (0.4 mg/mL) were orally administered from day 0 to 13 and *Agaricus* (0.5 mg/mL) from day 0 to 28. AeL: *Agaricus* (0.5 mg/mL) encapsulated liposomes (0.4 mg/mL) were also administered from day 0 to 28 ($n=7$).

with 1.0 mg/mL *A. blazei* Murrill water extract in the form of simple mixture served as drink, and (v) 1.0 mg/mL squid phospholipid liposome (PC/PS/Chol=9/1/10 molar ratio) with 1.0 mg/mL *A. blazei* Murrill water extract partially encapsulated served as drink. These drinks were orally administrated for 3 weeks. The orally administrated liposomal drink amount per mouse was approximately 5 mL per day. As shown in Fig. 3.5, *A. blazei* Murrill water extract alone and squid phospholipid alone served groups show moderate tumor suppression with total administrations being approximately 105 mg/mouse throughout the experimental term. When both *A. blazei* Murrill water extract and squid phospholipid were administrated simultaneously in a simple mixture form with the same amount of the individual components, boosting effect of squid phospholipid on sp2 myeloma cancer tumor suppression was observed. Similarly, when *A. blazei* Murrill water extract was partially encapsulated with squid phospholipid liposome with total administrations being the same amount with the above simple mixture form (105 mg/mouse), the boosting effect on cancer tumor suppression by *A. blazei* Murrill water

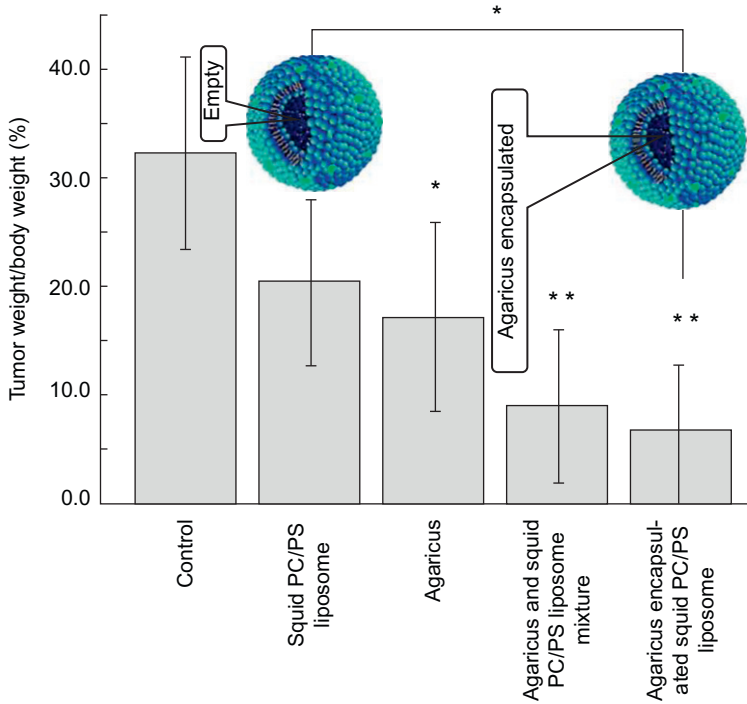

FIGURE 3.5 Effect of marine phospholipid based liposomes and *Agaricus* on tumor weight (%) against body weight of myeloma sp2-bearing BALB/c mice (Murakawa *et al.*, 2007). Myeloma sp2 tumor cells (1.5×10^5 cells/mice) were implanted into mice. Twenty days after implantation of the cells, liposomes (1.0 mg/mL), *Agaricus* (0.5 mg/mL), AaL: *Agaricus* (0.5 mg/mL)+liposomes (1.0 mg/mL) mixture, and AeL: *Agaricus* (0.5 mg/mL) encapsulated liposomes (1.0 mg/mL) were administered for 21 days. The results are the means±SD ($n=7$). Asterisks indicate significant difference as compared to control (*$p<0.05$, **$p<0.001$).

extract was more enhanced. Though there was no significant difference in tumor sizes between the simple mixture form administrated group, that is, group (iv) and the β-glucan partially encapsulated form administrated group, that is, group (v), the promotional effect seemed more superior in the form of encapsulated. In fact, although it was only one, the tumor-disappeared mouse was seen in the partially encapsulated form liposomal drink served group. If we succeed in encapsulating more amount of *A. blazei* Murrill water extract in the marine phospholipid liposomes, there should be a significant difference in tumor sizes between groups (iv) and (v). Thus, it was concluded that *A. blazei* Murrill water extract encapsulated marine phospholipid may be useful in myeloma sp2 therapy.

In the early 1990s, the mucoadhesive function of chitosan has received much attention for prolonging the residence time of dosage forms at the absorption site (Gupta *et al*., 1990). It has been reported that the stability of chitosan-coated liposomes in stimulated gastric fluid was significantly higher as compared to uncoated liposomes. It has been proposed that chitosan-coated liposomes might be utilized as a bioadhesive intestinal delivery system (Filipović-Grčić *et al*., 2001). Thus, it can be considered that chitosan-coated marine phospholipid may be beneficial in promoting antitumor activity of marine phospholipid or chitosan itself. It is anticipated that chitosan-coated marine phospholipid liposomes may also promote the anticancer effects by prolonging the retention on intestinal wall. Therefore, the tumor suppression effect of marine phospholipids with chitosan on myeloma sp2 tumor-bearing mice was investigated. Weanling BALB/c mice were divided into five groups. Control (only water served), 1.0mg/mL squid phospholipids liposome (PC/PS/Chol=9/1/10 molar ratio) alone served, 5.0mg/mL chitosan alone served, chitosan (5mg/mL)+squid phospholipid (1mg/mL) liposome mixture served, and chitosan-coated (5mg/mL) squid phospholipid liposome (1mg/mL) served were treated as groups (i), (ii), (iii), (iv), and (v), respectively.

The orally administered liposomal drink amount per mouse was approximately 5mL per day. Results (Hossain, 2007) showed that chitosan alone and squid phospholipid alone served groups showed slight tumor-associated weight loss (Fig. 3.6) and tumor suppressions (Fig. 3.7) throughout the experiment term. There was a significant cancer tumor-associated weight loss suppression (Fig. 3.6) as well as tumor suppression effect (Fig. 3.7) when both chitosan and squid phospholipid were administrated simultaneously in a simple mixture form and chitosan-coated liposome form. We may, thus, say that marine phospholipids not only promotes the effect of anticancer drugs and functional compounds but might also moderately suppress cancer-associated body weight loss.

V. BOOSTING EFFECT ON ANTIOBESITY COMPOUNDS

By inserting hydrophobic antiobesity compounds into the liposomal bilayers, marine phospholipids would boost the effect of the inserted hydrophobic antiobesity compounds. When marine phospholipids are served as liposomal drinks, they would be more effective than adding into solid foods or feeds. These facts were borne out by Okada *et al*. (2011). They carried out the following experiment. Brown seaweed (*Undaria pinnatifida*) lipid containing fucoxanthin (UL) encapsulated into scallop midgut gland phospholipids (PL) liposomes were prepared to see the promotional effect of marine phospholipid liposome on antiobesity. Animal model used in their study was 3-week-old male diabetic-obese

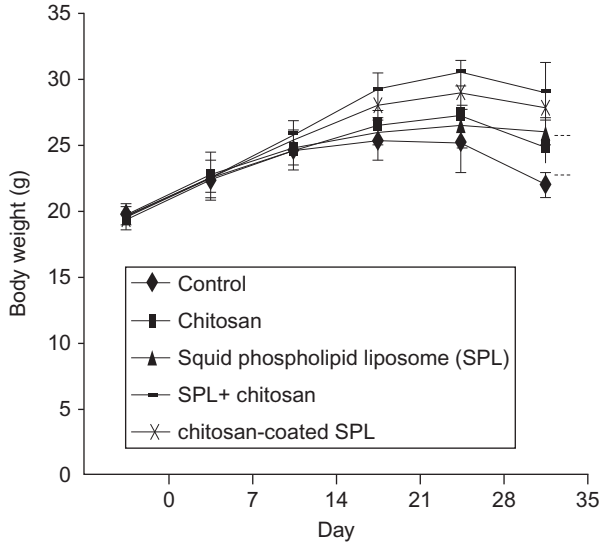

FIGURE 3.6 Effect of squid meal liposome and chitosan samples on the body weight of myeloma sp2-bearing BALB/c mice. Twenty days after implantation of the cells, liposomes (1.0 mg/mL), chitosan (5.0 mg/mL), chitosan (5.0 mg/mL)–liposomes (1.0 mg/mL) mixture, and chitosan-coated (5.0 mg/mL) liposomes (1.0 mg/mL) were administered for 35 days. The results are the means±SD (n=12). Asterisks indicate significant difference as compared to control and liposome (***$p<0.001$).

mice model KK-A^y mice. Each group received different combinations of lipid drinks, that is, 0.2% UL, 0.3% PL (scallop midgut gland phospholipid), 0.2% UL encapsulated 0.3% PL liposomes. The same amount of total intake of UL and PL in the form of feed were also set up for comparison. And as a result, the total intake of the PL and UL were almost the same between the drink form serving and feed form serving as illustrated in Fig. 3.8. Animals were sacrificed after a 4 weeks experimental feeding period, and adipose tissues and organs were dissected and then weighed. The mesenteric white adipose tissue (WAT) weight was reduced in all experimental groups as compared to the control group. This reduction was significantly different ($p<0.05$) from the control group for mice receiving the UL-encapsulated PL liposomal drink. Total WAT is composed of perirenal WAT, retroperitoneal WAT, mesenteric WAT, epididymal WAT, and gluteal WAT. Only the UL-encapsulated PL liposome (9.02±1.23 g/100 g BW) exhibited a significant weight reduction of the total WAT compared to the control group (10.1±0.41 g/100 g BW). Uncoupling protein 1 (UCP1) mRNA expression levels and UCP1 expression were determined by RT-PCR (real-time polymerase chain reaction) analysis and Western blotting analysis, respectively. UCB1 is known to

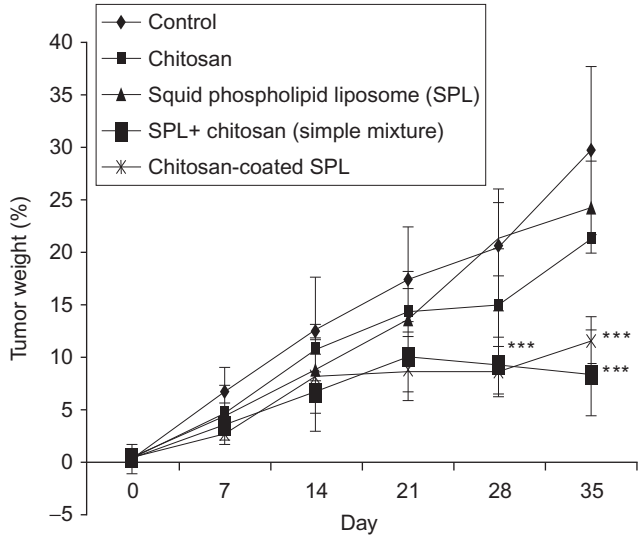

FIGURE 3.7 Effect of squid meal phospholipid liposome and chitosan samples on the tumor weight of myeloma sp2-bearing BALB/c mice. 20 days after implantation of the cells, liposomes (1.0 mg/mL), chitosan (5.0 mg/mL), chitosan (5.0 mg/mL)+liposomes (1.0 mg/mL) mixture, and chitosan-coated (5.0 mg/mL) liposomes (1.0 mg/mL) were administered for 35 days. The results are the means±SD (n=12). Asterisks indicate significant difference as compared to control and SPL (***p<0.001).

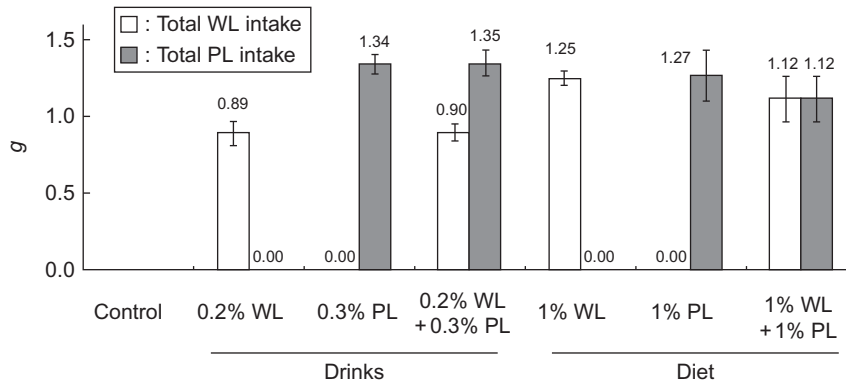

FIGURE 3.8 Total sample intake of KK-A^y mice during the experimental period. For the abbreviations, refer to the text.

decrease WAT by being upregulated to generate heat. Both UCP1 (Fig. 3.9) and UCP1 mRNA expression (Fig. 3.10) in epididymal fat on mice fed with the UL-encapsulated PL liposomes were significantly higher than in the control group. Although the both groups receiving

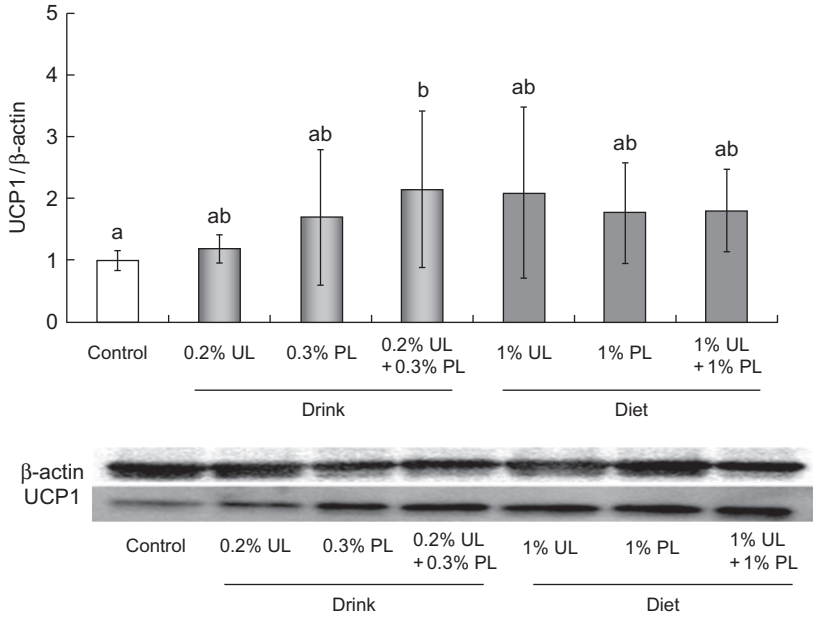

FIGURE 3.9 Western blotting analysis of uncoupling protein 1 (UCP1) in epididymal WAT and relative expression level of UCP1 protein compared to β-actin (Okada *et al.*, 2011). Columns with different superscript letters are significantly different ($p<0.05$).

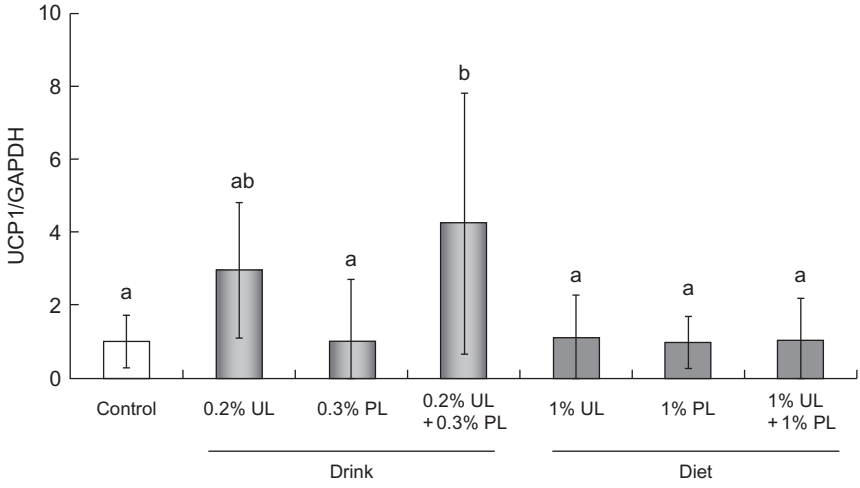

FIGURE3.10 UCP1 mRNA expression levels in epididymal WAT (Okada *et al.*, 2011). Expression of UCP1 mRNA was estimated by quantitative real-time polymerase chain reaction. Relative values were presented as the ratio of UCP1 mRNA to GAPDH mRNA.

0.2% UL and the 0.2% UL-encapsulated 0.3% PL liposome groups exhibited higher UCP1 mRNA expression levels than the control, these levels were significantly different only in the liposome served group being 4.23 times higher than that of control group. These results suggest that UL and PL work in an additive manner to reduce obesity, resulting in a greater antiobesity effect than that which would be expected at these reduced concentrations. And the PL (the marine phospholipid) liposomal form must be more effective than the feed form in serving to exert antiobesity function. Though fucoxanthin is no doubt an excellent functional compound to reduce WAT, limited supply has been a bottle neck. The supply of β-glucan is also limited. Marine phospholipids especially in the form of liposomes should help to boost the effect of precious functional compounds such as it has been seen.

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