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More Efficient Utilization of Fish and Fisheries Products

MEDICAL APPLICATIONS OF FISHERIES BY-PRODUCTS

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Abstract

The *sn*-2 position docosahexaenoic acid inserted phospholipids (*sn*-2 DHA-PL) are beginning to receive attentions because of their beneficial functions. There are two alternatives to obtain *sn*-2 DHA-PL. Squid skin, muscle and testis of late run salmon, fish roe are by-products or wastes that can be subjected to extract crude *sn*-2 DHA-PL. High purity *sn*-2 DHA-PL can be obtained through phospholipase A_2 mediated esterification of highly purified DHA into soy lysolecithin. The obtained high purity *sn*-2 DHA-PL showed promotional effects on leukemia cell differentiation when retinoic acid or dibutyryl cyclic adenosine monophosphate was used as a differentiator. When *sn*-2 DHA-PL, a mixture of *sn*-2 DHA-phosphatidylcholine and *sn*-2 DHA-phosphatidylserine were made into liposomes, they showed antitumor activity against Meth-A fibrosarcoma both *in vitro* and *in vivo*. Highly branched glycogen obtained from scallop and a novel peptide glycan from squid ink, also showed antitumor activities against Meth-A fibrosarcoma. Squid pen β-chitin laminated with salmon skin collagen was borne out to be a favorable artificial human skin. DNA from salmon testis could apply to antibacterial film with silver ion. All materials presented here are no doubt highly bioavailable, and should thus become applicable to varieties of medical uses.

1. INTRODUCTION

To decrease by-products and wastes from bioresources, various ways of utilizations from highly value added products to a fairly low value added but with large mass demand must be developed. At present, the only available utilization of by-products and wastes from marine sources in a large scale has been the production of feeds. However, markets for feeds are limited. For this reason, developing a more effective way to utilize fisheries related by-products and wastes are becoming more and more important.

In this article, components contained in the by-products and wastes from salmon, squid and scallop that may be applicable to medical uses are demonstrated, since these three species are consumed in an enormous amount in the world. And vast excess by-products

and wastes from these three species have been a burden to seafood processing companies. Equipment investment for reducing by-products and wastes might become possible if we could develop value added products. Medical uses should have the potential to be the most highly value added, and expected to be consumed in large amounts, though it is very hard to realize for the time being.

The purpose of this article is to show the potential benefits of utilizing phospholipids, glycogen, peptide glycan, chitin and DNA contained in the by-products and wastes from salmon, squid and scallop.

2. THE *sn*-2 DOCOSAHEXAENOIC ACID INSERTED PHOSPHOLIPIDS

Benefits of taking docosahexaenoic acid (DHA) are well recognized. Hara [1] pointed out that incorporated DHA must be converted to phospholipids before exerting its biological functions. In the last decade, it has been shown through many studies, both in vivo and in vivo, that DHA inserted phospholipids, especially the *sn*-2 DHA inserted phospholipids (*sn*-2 DHA-PL) have many functional benefits other than the health benefits of DHA itself. For example, the *sn*-2 DHA-PL is known to enhance survivals of tumor bearing mouse [2] and to promote cell differentiation of erythroleukemia cancer cells [3]. We also demonstrated that *sn*-2 DHA-PL liposomes with 70% phosphatidylcholine (PC) and 30% phosphatidylserine decreases tumor size of the Meth-A fibrosarcoma bearing mice without any immunomodulator encapsulated (Fig. 1) [4]. In vitro study done at the same time designated that those liposomes decrease the viability of the Meth-A fibrosarcoma (Fig. 2) and increase phagocytic effect of macrophage like J774-1 cells and also slightly increase the number of the phagocytic activated cells (Table 1). Another functional benefit of *sn*-2 DHA-PL is the promotional effect of cell differentiation. Suzuki [3] and his coworkers showed that *sn*-2 DHA-PL induces cell differentiation of embryo. We have been bearing out that the *sn*-2 DHA-PL may be beneficial for therapy of leukemia, because it promotes the effect of HL-60 cell (the cell line of leukemia) differentiators such as all *trans* retinoic acid [5]. Differentiated HL-60 cells become mortal and soon they disappear.

Compared with the *sn*-2 DHA-PC, *sn*-2 DHA inserted phosphatidylethanolamine (*sn*-2

Table 1
Phagocytic effect of macrophage like J774-1 cells induced by *sn*-2 DHA PC/PS* liposomes

Concentration	Phagocytic activity			
(μg/mL)	(A)	(%)	(B)	(%)
0	47.4	100.0	8.5	100.0
10	64.4	135.9	9.5	112.3
25	82.1	173.2	10.1	119.3

(A), Number of phagocytic cells/ 300cells ; (B), Number of beads/Individual phagocytic cells.
*Same abbreviation as in Fig. 1.

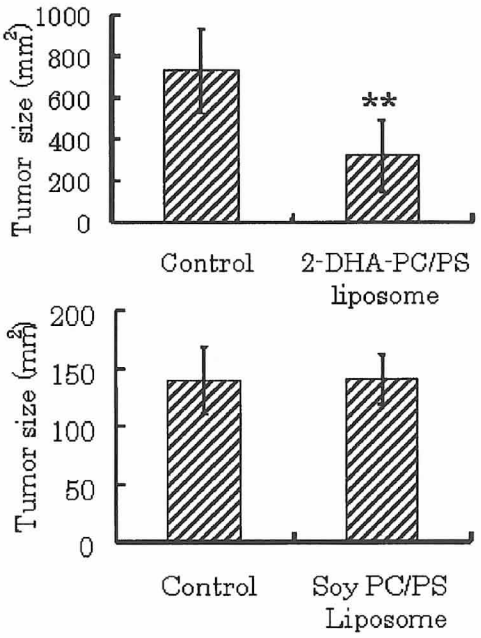

Figure 1. Antitumor effect of *sn*-2-DHA–PC/PS* liposome on Meth-A fibrosarcoma bearing BALB/c mice. **sn*-2 DHA inserted phospholipids with 70% phosphatidylcholine (PC) and 30% phosphatidylserine (PS) in mol%. ***p*<0.01 vs. control. Fujimoto, A. et al. (2001) [4].

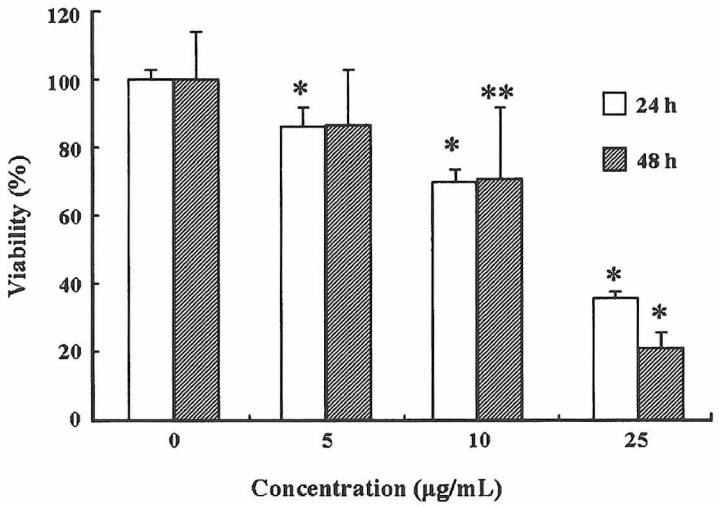

Figure 2. Cytotoxic effect of *sn*-2 DHA–PC/PS liposomes on Meth-A fibrosarcoma. Data are shown as means ± S.D. (n=6). **p*<0.01, ***p*<0.05 vs. 0 μg/mL. Fujimoto, A. et al. (2001) [4].

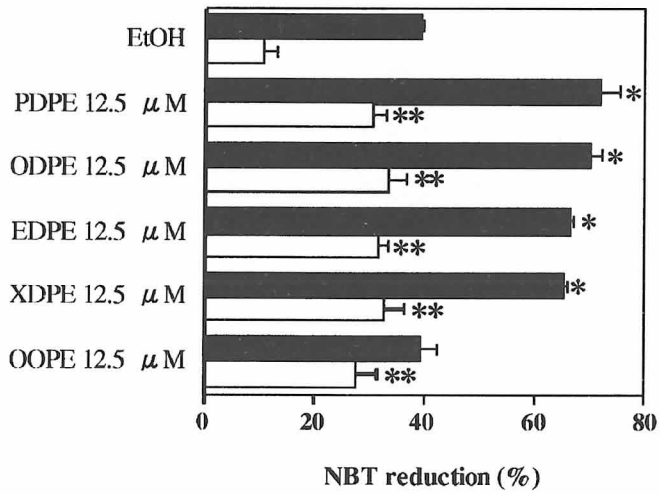

Figure 3. Effect of individual *sn*-2 DHA-PE molecular species on differentiation of HL-60 cells. Values represent means ± S.D. (n=3). *p<0.01 vs. control (retinoic acid (RA) added, dark bar); **p<0.01 vs. control (RA free, white bar). Tochizawa, K. et al. (1997) [5].

DHA-PE) was more effective on differentiating HL-60 cells. And as shown in Fig. 3, HL-60 cells coincubated with 12.5μM *sn*-2 DHA-PE and 100nM retinoic acid doubled the nitro blue tetrazolium reduction-positive cell number (an indicator of HL-60 cell differentiation) compared to those with 100nM retinoic acid alone [5]. We also demonstrated that a mixture of *sn*-2 DHA-PE and *sn*-2 EPA-PE obtained from natural sources such as late run chum salmon muscle, testis, and roe have promotional effects on retinoic acid induced (data not shown) or dibutyryl cyclic adenosine monophosphate (Bt^2-cAMP) induced HL-60 cell differentiations (Fig. 4) [6]. Squid skin is known to contain an extremely DHA enriched *sn*-2 DHA-PL (Table 2) [7]. There is no doubt that this PL can promote retinoic acid or Bt^2-cAMP induced cell differentiation. The reasons why *sn*-2 DHA-PL can promote cell differentiation are under investigations by the authors and other groups.

Recently, Inoue [8] reported that among the supplemented oils which are squid skin PL, egg yolk PL, and fish triacylglycerol, squid skin PL supplemented diet was the only available lipid to prevent apoplexy of SHR-SP rats as illustrated in Fig. 5. It was suggested that DHA should be bound with glycerophospholipid backbone, but not with glycerol backbone to exert this noticeable functionality.

3. HIGHLY BRANCHED GLYCOGEN

The existence of an antitumor fraction in a hot water extract from scallop was first reported by Sasaki et al. in 1978 [9]. And in 1998, Takaya and his coworkers [10] bore out that highly branched glycogen was the one that cured the Meth-A fibrosarcoma

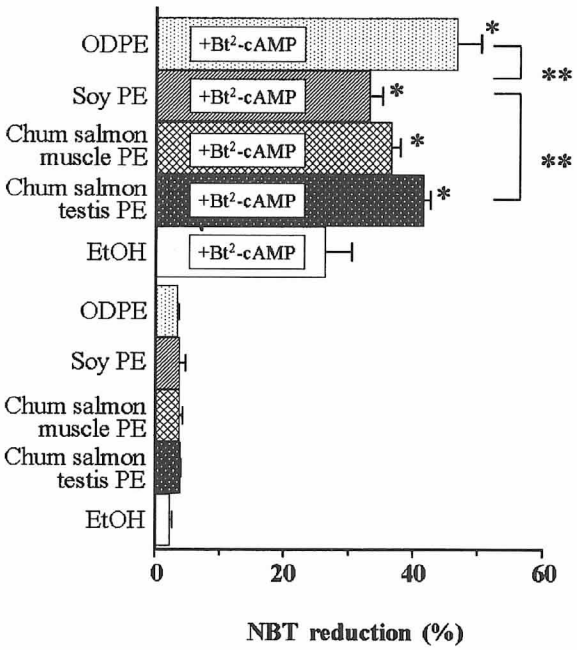

Figure 4. Comparison of various PEs on differentiation of HL-60 cells induced by Bt^2-cAMP. HL-60 cells (5×10^4 cells/mL) were incubated with 100 μM Bt^2-cAMP and/or 50 μMP PEs for 72 h after preincubation for 24 h. Data are shown as means ± S.D. (n=3). *$p<0.01$ vs. Bt^2-cAMP-treated cells; **$p<0.01$ vs. soy PE + Bt^2-cAMP-treated cells. Hosokawa, M. et al. (2001) [6].

implanted mice. Plant for isolating this glycogen has been designed by Aomori Advanced Industrial Technology Center in Japan.

4. A NOVEL ANTITUMOR PEPTIDOGLYCAN

Matsue et al. [11] discovered that squid ink from *Illex argentinus* has an antitumor activity. They found that a novel peptidoglycan with fucose as shown in Fig. 6 was the one that exerts the antitumor activity through modifying the biological response of the phagocytic activity of macrophages [12]. As shown in Figs. 7 and 8, both the unheated and heated novel peptidoglycans show antitumor effect against Meth-A tumor cells [12].

5. SQUID PEN β-CHITIN LAMINATED WITH SALMON SKIN COLLAGEN AS AN ARTIFICIAL HUMAN SKIN

Compared to a well known α-chitin from crab, squid pen β-chitin is much softer even

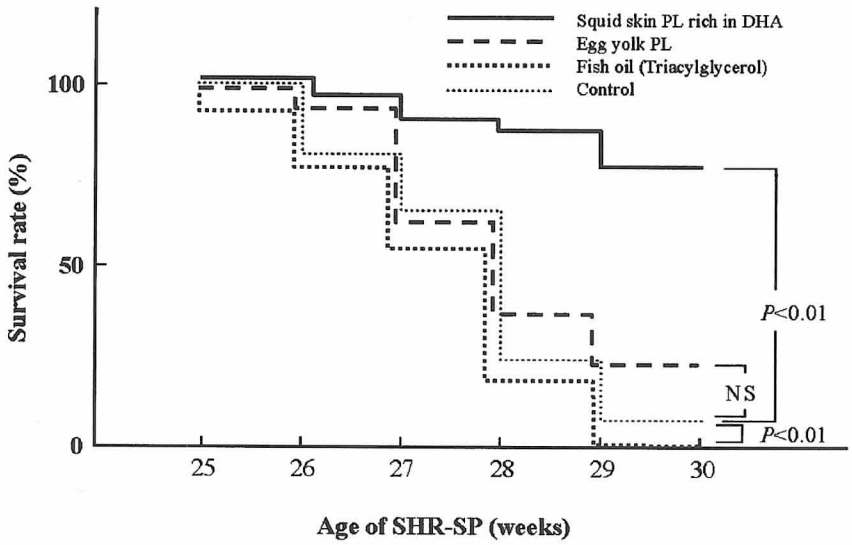

Figure 5. Effect of feeding diet containing various types of lipids on survival of SHR-SP rats. PL, phospholipids. Inoue, Y. (2001) [8].

Table 2
Fatty acid composition of egg yolk phospholipid (PL), DHA-enriched egg yolk PL obtained from fish oil-fed hens, and squid skin PL

	16:0	18:0	18:1	20:4	22:6
Egg yolk PL	31.2	15.3	26.6	4.9	3.5
DHA yolk PL	35.2	10.7	24.5	1.5	10.9
Squid PL	27.0	7.8	2.7	2.7	33.3

Ono, M. et al. (1997) [7]

though strength against tearing is comparable to each other (Table 3) [13]. For this reason, squid pen β-chitin laminated with salmon skin collagen must be a favorable artificial human skin. In fact, after culturing fibroblasts on that laminated sheet, proliferation of those cells immediately occurred and covered the whole sheet within 8 days as shown in Fig. 9 [14]. DNA labeling index which is a marker for the occurrence of cancer was low throughout the incubation period of the fibroblasts proliferation on the salmon skin collagen laminated squid β-chitin sheet (data not shown) [13].

6. SALMON TESTIS DNA-ALGINIC ACID FILM

Iwata and his coworkers [15] invented a DNA-alginic acid film by mixing up DNA sodium salt and sodium alginate solution, then cast in a sheet form, dry and treated with

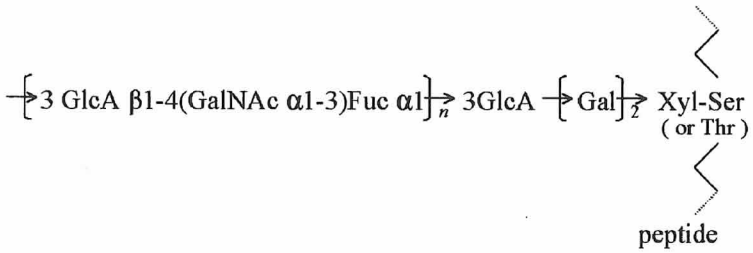

Figure 6. Antitumor peptidoglycan with novel carbohydrate chain obtained from squid (*Illex argentinus*) ink. Fuc, fucose; GlcA, glucuronic acid; GalNAc, N-acetylgalactosamine. Matsue, H. et al. (1997) [11].

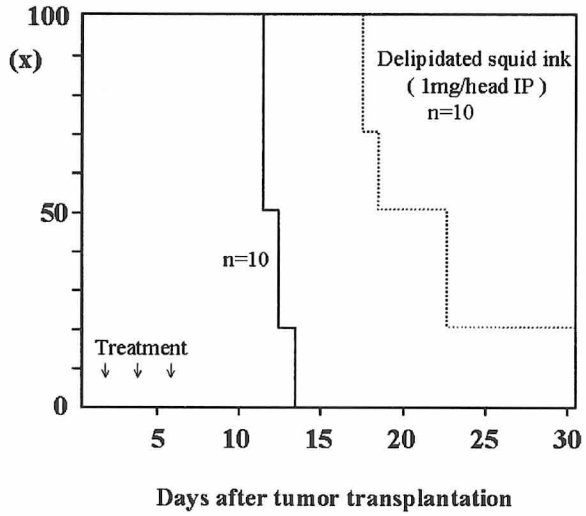

Figure 7. Anti-tumor activity of delipidated squid ink. Meth-A tumor cells (2×10^4) were intraperitoneally (IP) transplanted into mice. Mice were IP treated with 1mg/mL/head of delipidated ink, three times on days 2, 4 and 6 after tumor transplantation. Sasaki, J. et al. (1997) [12].

calcium chloride. The sheet rapidly absorbed ethidium bromide, a well-known carcinogenic compound. Therefore, this filter might be applicable not only to the medical uses, but also to variety of fields.

Kitamura and his coworkers [16] developed a salmon testis DNA-alginic acid film to impregnate silver ion. This was successfully done merely by mixing up silver nitrate before casting. As shown in Table 4, the silver ion impregnation efficiency of the DNA-alginic acid film was about five times superior than the DNA free alginic acid film. And for this reason, this novel silver-DNA-alginic acid sheet may be useful for antibacterial purposes.

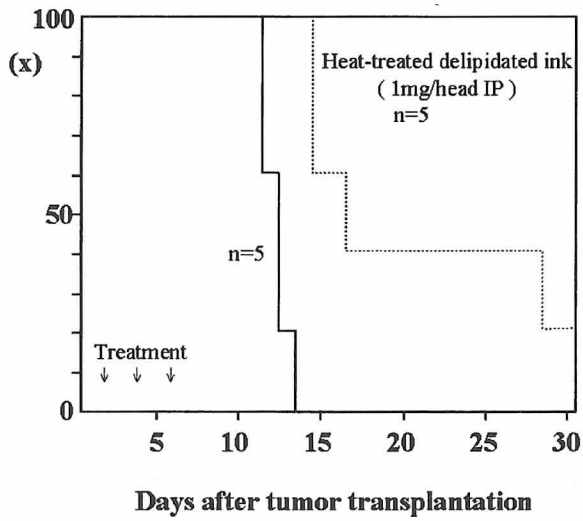

Figure 8. Anti-tumor activity of delipidated squid ink after heat treatment at 100°C for 10min. Meth-A tumor cells (2×10^4) were intraperitoneally (IP) transplanted into mice. Mice were IP treated with 1mg/mL/head of heat-treated delipidated ink, three times on days 2, 4 and 6 after tumor transplantation. Sasaki, J. et al. (1997) [12].

Table 3
Comparison of properties of chitin sheets between squid and crab

	Stiffness	Burst strength ($kP\cdot m^2/g$)	Braking strain (km)	Expansibility (g/m^2)
Squid chitin sheet	12	6.9	6.9	21.9
Crab chitin sheet	66	3.8	7.1	21.9

Takai, M. et al. (1995) [13]

7. FUTURE VIEW

So far, medically beneficial components from wastes of abundantly supplied species e.g. salmon, squid and scallop, have been introduced. Unfortunately, most of the studies other than squid β-chitin-salmon collagen sheet, an artificial skin, still remain in the low phases of development for practically medical uses. However, there is no doubt that phospholipids, glycogen, peptidoglycan, chitin, DNA and alginic acid have high bioavailability.

Antitumor active compounds depicted here are so called biological response modifiers (BRM). BRM is known to make immune systems more active. Side effects of antitumor drugs are expected to be minimum when using these BRM. The *sn*-2 DHA-PL is considered to be another BRM. It may make the cancer cells more sensitive to drugs. For this reason, if the *sn*-2 DHA-PL is preloaded prior to the anti cancer drugs, it may be

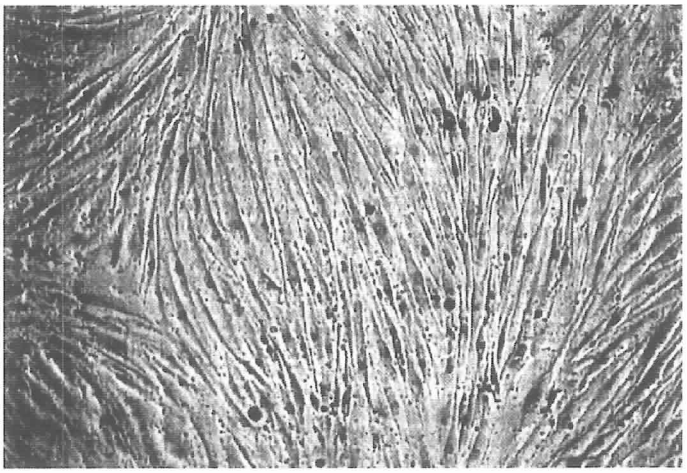

Figure 9. Proliferation of fibroblast on salmon skin collagen laminated squid β-chitin sheet. Takai, M. (1996) [14].

Table 4
Antibacterial activity of alginate (AL) film with and without Salmon testis DNA as a carrier of silver ion (Ag) in relation to the Ag impregnation

	AL-Ag	DNA-AL-Ag
Ag(μg)	4.21	21.7
E. coli	89*	226*
S. aureus	387*	708*

*Inhibition area (mm^2) of cell-growth. Kitamura, H. et al., (1997) [16]

expected to reduce the side effects of the drugs by reducing their dose amount. To increase the quality of life of patients, and to give intensive to reduce wastes, or to consume byproducts, it should be very important to study the BRM contained in those sources.

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