Liposomal *sn*-2 DHA inserted phospholipid decreases tumor size of fibrosarcoma

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***KEY WORDS*: phospholipid, liposome, fibrosarcoma, phagocytosis, antitumor**

INTRODUCTION

Liposomes especially the multilamella vesicles (MLV) are known to be phagocyted by macrophages. This was considered to be a problem when designing a drug delivery system. If we take another way of looking at this problem, we could take advantage of this draw back of MLV. For instance, by encapsulating a biological response modifier, we may activate macrophages. The *sn*-2 docosahexaenoic acid (DHA) inserted phospholipids (2-DHA-PL) have been receiving attentions on their boosting effects on functional compounds such as retinoid or hormones without any serious side effect. For this reason, it is expected to enhance therapeutic effects of the encapsulated compound. In this study, we evaluated the antitumor activity of 2-DHA-PL liposome against Meth-A fibrosarcoma both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials

Meth-A fibrosarcoma cells (RCB 0464) were purchased from Riken cell bank (Ibaraki, Japan) and Macrophage-like J774-1 cells were generously supplied by Cell Resource Center for Biomedical Research, Tohoku University (Miyagi, Japan). RPMI 1640 medium was purchased from Giboco (Grand Island, New York), and fetal bovine serum (FBS) was from ICN Biomedicals Inc. (Costa Mesa, CA). WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) and 1-methoxy PMS (1-Methoxy-5-methyphenazinium methylsulfate) were obtained from Dojindo laboratories (Kumamoto, Japan).

Preparation of phospholipids and liposome

sn-2-Docosahexaenoyl phosphatidylcholine (2-DHA-PC) was enzymatically synthesized from soy lysophosphatidylcholine (LPC) via phospholipase A_2 -mediated esterificaton as previously reported.[1] Then, *sn*-2 DHA-phosphatidylserine (2-DHA-PS) was prepared from 2-DHA-PC through phospholipase D-mediated transphosphatidylation.[2] Liposomes with 7/10 of 2-DHA-PC and 3/7 of 2-DHA-PS were prepared according to the Proliposome method patented by Lucas Meyer Inc[3]. Then, by passing the liposome through a 1 μm pore size membrane filter (AVESTIN Inc.) and by adjusting the final concentration to 1 mg/mL, uniformed size liposomes were obtained.

Mice and tumor type

Female BALB/c mice, weighing ca. 20 g, were used. The Meth-A fibrosarcoma used was maintained in our laboratory in an ascitic form. Tumor cells (2.7×10^6 cells/animal) suspended in Hanks' balance salt solution (GIBCO, Grand Island, NY) were implanted intraperitoneally into the mice .

Assay of antitumor activity

The liposomes were injected intraperitoneally at dosages of 1 mg/mouse on days 2, 4, and 6 after tumor implantation. Mice receiving similar treatment with PBS alone served as controls. The mice were observed for 3 weeks and tumor size of those mice were measured.

Cell culture

Meth-A fibrosarcoma cells and macrophage like J774-1 cells were cultured in RPMI 1640, supplemented with 10 % heat-inactivated (FBS), 2 mM L-glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin at 37 ℃ in a 5 % CO2 humidified incubator.

WST-1 dye reduction assay

Meth-A fibrosarcoma cells were seeded in a 96-well plate (Iwaki, Tokyo) with 200 μL growth medium to give 1.0×10^3 cells. Then, liposomes were added. And after 24 h, macrophage like J774-1 cells were added. Cell viability was determined every 24 h following the WST-1 dye reduction assay with slight modification. The medium was not removed, and the cells were cultured for another 0-48 h in the medium (200 μL). WST-1 (3.3 mg/mL PBS included 7 % 1-metoxy PMS) was added to each well (20 μL / 200 μL medium), and the plate was incubated at 37 ℃ for 3 h. Absorbance at 450 / 650 nm was measured for each well on a Micro plate reader.

Phagocytic activity assay

Macrophage like J774-1 cells were seeded in a 12-well plate (Iwaki, Tokyo) with 1mL growth medium to give 5.0×10^4 cells. And after 24 h, liposomes were added. Further after 24 h, 1.75 μm beads (2.5×10^6) were added. The medium was not removed, and the cells were cultured for another 24 h in the medium (1mL). Then, the cells were rinsed with PBS and incorporated beads were counted.

RESULTS

2-DHA-PL liposome showed cell growth inhibition, depending on each concentration, against Meth-A fibrosarcoma *in vitro* (Fig.1, A) while 2-DHA-PL liposome enhanced phagocytic activity of macrophage like J774-1 cells and slightly increased those cell number *in vitro* up to 2.5 μg/mL level of addition after 48 h (Table 1 and Fig.1, B). Phagocytic activity was enhanced especially at 2-DHA-PL liposome concentrations of 10 and 25 μg/mL (From 153.85 to 175.69 %). Soy phospholipid liposome (soy PL liposome) also enhanced phagocytic activity depending on each concentration though the effect was less than 2-DHA-PC.

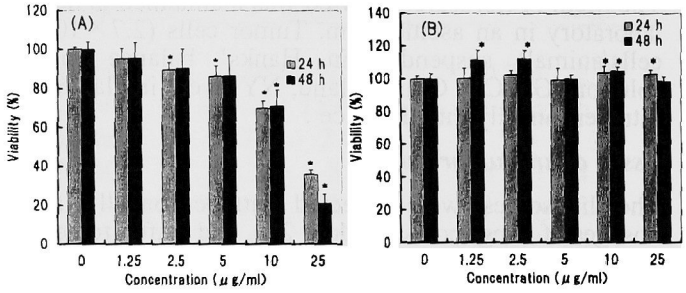

Fig. 1 Effect of 2-DHA-PC/PS liposome on Meth-A fibrosarcoma cells and macrophage like J774-1 cells.
(A) Meth-A fibrosarcoma (B) Macrophage like J774-1
Data are shown as means ±S.D. (n=6) *P<0.01 **P<0.05 vs. control.

Table 1. Phagocytic effect of macrophage like J774-1 cells induced with 2-DHA-PC/PS liposome after 48 h

Concentration	2-DHA-PC/PS liposome treatment			
μg/mL	(A)	(%)	(B)	(%)
0	55.00	100.00	12.07	100.00
5	60.00	109.09	13.36	110.68
10	84.62	153.85	13.95	115.62
25	96.63	175.69	15.22	126.14

Concentration	Soy PC/PS liposome treatment			
μg/mL	(A)	(%)	(B)	(%)
0	55.00	100.00	12.07	100.00
5	56.25	102.27	12.02	99.56
10	74.32	135.14	11.85	98.16
25	78.26	142.29	11.88	98.48

(A) Number of phagocytic cells / 300cells × 100%
(B) Number of beads / individual phagocytic cell

Soy PL liposome did not show any cell growth inhibition (data not shown). And it did not increase the phagocytic cell number as shown in Table 1.

A noticeable feature was that 2-DHA-PL liposome decreased the tumor size of Meth-A fibrosarcoma-bearing mice to about 50% (Fig. 2).

DISCUSSION

Liposomes with polyunsaturated fatty acid inserted phospholipids have been said that they are less stable. And for this reason, they permits leakage of the encapsulated substances compared to a saturated fatty acid containing phospholipids.[4] For this reason, saturated fatty acid containing phospholipids have been generally accepted as materials for liposomes. However, 2-DHA-PL have many beneficial functions such as boosting effect on retinoid or hormone functions without any serious side effect.[5,6]

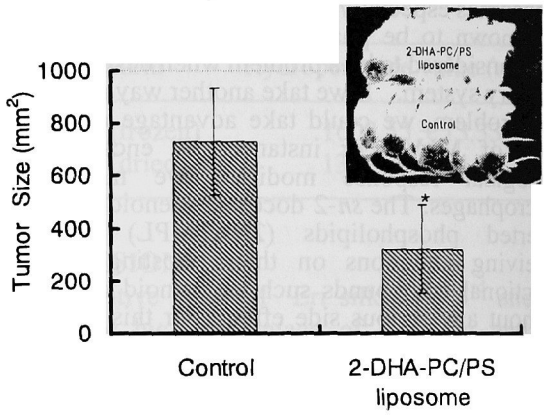

Fig. 2 Antitumor effect of 2-DHA-PC/PS liposome on Meth-A fibrosarcoma bearing BALB/c mice.
Data are shown as means ±S.D. (n=6) *P<0.01 vs. control.

In this study, we realized that 2-DHA-PL liposomes are not unstable as predicted (data not shown). Growth inhibition of 2-DHA-PL liposomes against Meth-A fibrosarcoma cells and phagocytic enhancement of 2-DHA-PL liposomes on macrophage like J774-1 cells were superior to that of soy PL liposomes. It is often reported that enhance activity of immunocyte such as macrophage relates with antitumor.[7] Thought our study, we considered that the decrease in tumor size was attributed to the increase in phagocytic activity of the individual macrophage in addition to the increase in that cell number, and also to a direct suppression against tumor cell growth.

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