ω-3 Fatty Acid–Containing Liposomes in Cancer Therapy (43943)

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Abstract. **ω-3 fatty acids are associated with reduced growth and incidence of certain cancers, and in this report we demonstrate that a fish oil diet (rich in ω-3 fatty acids) enhances the longevity of mice bearing the myeloid leukemia T27A. We have proposed that the ω-3 fatty acid docosahexaenoic acid (DHA, 22:6$^{\Delta 4,7,10,13,16,19}$) may induce structural changes in tumor cell plasma membranes resulting in reduced tumor growth *in vitro*. Here, we test whether liposomes containing DHA (18:0, 22:6 PC) have antitumor effects *in vivo*, leading to enhanced longevity of the tumor-bearing host. Male BALB/c mice (6–8 weeks old) were inoculated intraperitoneally with a T27A tumor dose known to cause 100% mortality of syngeneic (BALB/c) mice in less than 2 weeks. Small unilamellar vesicles (liposomes) were prepared, composed of phosphatidylcholine (PC) with 18:0 in the *sn*-1 position and one of the following fatty acids in the *sn*-2 position: 18:0, 18:1ω9 (oleic), 18:3ω3 (α-linolenic), 20:4ω6 (arachidonic), 22:6ω3 (docosahexaenoic). The liposomes were injected intraperitoneally into tumor-bearing mice at various times: concurrently with the tumor inoculum, at select times during tumor growth, and when the mice were moribund. Mouse survival was then charted. DHA-containing lipid vesicles (18:0, 22:6 PC) caused a statistically significant increase in survival of the tumor-bearing mice when compared with 18:0, 18:1 PC. Lipid vesicles of 18:0, 18:0 PC showed no benefit, and 18:0, 20:4 PC was not significantly different than 18:0, 18:1 PC. Lipid vesicles containing a different ω-3 fatty acid, 18:0, 18:3 PC, also effectively enhanced tumor-bearing mouse survival. The greatest benefit was achieved if either the liposome treatments were spaced throughout the tumor growth period, or if the tumor inoculum was suspended in the liposome preparation (without further liposome treatments). Neither lipid peroxidation nor prolonged inflammatory responses appeared to be pertinent, leaving membrane structural changes as a feasible mode of liposome action. With antitumor properties of their own, ω-3 fatty acid-containing lipid vesicles may offer an important new avenue in combination cancer therapies.** [P.S.E.B.M. 1995, Vol 210]

ω-3 fatty acids have recently garnered the interest of scientists and laypersons because of the fatty acids' purported health benefits in various diseases, including cancer. These fatty acids are named for their characteristic double bond three carbons from the methyl (ω) end of the acyl chain. Docosahexaenoic acid (DHA), 22:6$^{\Delta 4,7,10,13,16,19}$, is the longest and most unsaturated ω-3 fatty acid found in significant amounts in biological systems. It is known to impart to artificial and natural membranes structural changes such as increased permeability (1, 2), fusogenicity (3), and altered expression of membrane epitopes (4–6).

Although present at high concentration in only select tissues such as rod outer segment, brain, and sperm, DHA may be incorporated readily into both normal and neoplastic tissues where it persists (1, 6–8). We have shown that the murine leukemia T27A incorporates DHA from either dietary sources or culture medium into membrane phospholipids (4; Zerouga M *et al.*, submitted). In culture, DHA is assimilated into phospholipids with the following preference: phosphatidylethanolamine ⩾ phosphatidylcholine (PC) > phosphatidylserine + phosphatidic acid, phos-

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phatidylinositol (Zerouga M *et al.*, submitted). Although DHA is naturally metabolized into phospholipids of living cells, excessive DHA levels in culture medium will reduce T27A cell growth (Zerouga M *et al.*, submitted). Furthermore, fusion of lipid vesicles composed of DHA-containing PC (18:0, 22:6 PC) to T27A cells is associated with reduced cell viability (5), perhaps stemming from the increased permeability of tumor cell plasma membranes after 18:0, 22:6 PC fusion (2). This implies that, under certain conditions, DHA has anticancer properties that may be exploited to develop therapeutic regimens.

The therapeutic potential of DHA-containing liposomes depends upon the demonstration that DHA's tumor toxicity shown *in vitro* also occurs *in vivo* without untoward effects on the whole organism. Here, we report on the effects of DHA-containing PC lipid vesicles on the survival of tumor bearing mice. We compare the efficacy of PCs with stearic acid (18:0) in the *sn*-1 position and various fatty acids (stearic; oleic, 18:1ω9; α-linolenic, 18:3ω3; arachidonic, 20:4ω6; DHA, 22:6ω3) in the *sn*-2 position. (Phospholipids typically contain a saturated fatty acid in the *sn*-1 position and an unsaturated fatty acid in the *sn*-2 position.) We also test schedules for lipid vesicle administration, from Day 0 (in the tumor inoculum) to the moribund animal, and explore whether dietary DHA has anticancer effects in the tumor-bearing host. The results of these experiments have importance for designing chemotherapies and natural anticancer regimens that utilize DHA.

Materials and Methods

Tumor Line. T27A, a murine leukemia isolated from a BALB/c mouse infected with N-tropic Friend murine leukemia virus (9, 10), was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This cell line is not widely described, and therefore we have shown pertinent characteristics of the line in Table I. Prior to and during this study, the ascites tumor was maintained by weekly passage into syngeneic hosts. For the experimental procedures, the tumor (≥99% viable) was inoculated into syngeneic mice by intraperitoneal injection of 100 μl of sterile Hanks' balanced salt solution containing 1×10^4 T27A cells (i.e., 1×10^5 cells/ml). In one set of experiments, the T27A cells were resuspended in liposome preparations at 2×10^4 cells/ml, and 0.5 ml of the T27A-liposome mixture (1×10^4 cells) was injected intraperitoneally.

Mice. BALB/c male mice, ~6 weeks old, were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and housed in the fully AAALAC-accredited animal facility in the IUPUI School of Science. Mice were housed communally in groups of four or five, and allowed water *ad libitum*. In most experiments, the mice were allowed unrestricted access to a standard chow (Purina rodent diet #5001). Animals on experiment diets (described below) were given twice their usual food consumption at the start of the dark cycle (7 PM–7 AM) and the uneaten food was removed the following morning to avoid rancidification. Upon arrival from the supplier, the 6-week-old mice were enrolled on the experimental diets, and continued on the diets until their death. This dietary regimen has been previously described (4).

Table I. Characteristics of the T27A Leukemia Line

Characteristic[a]	Source
Surface markers present	
MHC class I	This study and Ref. 9
MHC class II	Ref. 9
LFA-1	This study
Surface markers absent	
Thy-1	Ref. 9
sIgM + sIgG	This study and Ref. 9
Equivocal surface marker expression[b]	
CD4	This study
CD8	This study
Hemoglobin not inducible with DMSO[c]	Ref. 10
~15 hr generation time in vitro	This study
Tumor-bearing mice succumb within 10 days[d]	This study

[a] The following monoclonal antibodies (specificity) were used in this study to identify surface markers: SF1-1.1.1 (K^d), 34-5-8S (D^d), M1/42.3.9.8 (monomorphic H-2 class I epitope), 15-5-5S (D^k, $K^{d,f}$); H129.19 (CD4); 53.6.7, 2.43 (CD8), M17/5.2, M17/4.4.11.9 (LFA-1).
[b] Approximately 20% of cultured T27A cells stained with anti-CD4 moncolonal antibody (H129.19), and approximately 50% stained with anti-CD8 (monoclonal antibodies 53.6.7 and 2.43).
[c] Many Friend murine leukemia virus-induced leukemias appear to be erythroid in origin based in part on hemoglobin production (9, 10).
[d] 100% mortality of syngeneic mice inoculated intraperitoneally with 1×10^5 viable T27A cells adapted to this passage regimen.

Experimental Diets. Modified AIN-76 diets were purchased from ICN Nutritional Biochemicals (Cleveland, OH). The oil content of the diets included 0.5% corn oil for essential fatty acids and an additional 10% oil as corn oil (CO), hydrogenated coconut oil (HCO), or menhaden oil (MO, a generous gift of Zapata-Haynie Corp., Reedville, VA). The fatty acid contents of the oils are shown in Table II. DL-α-tocopherol acetate was present at 0.22 g/kg diet as an antioxidant.

Lipid Vesicles. Distearoyl and mixed chain phosphatidylcholines were purchased from Avanti Polar Lipids (Alabaster, AL) and stored in hexane at −20°C. Appropriate amounts of the lipids were pipetted into glass tubes, dried under a stream of nitrogen, and desiccated under a strong vacuum for 2 hr. The lipids were rehydrated in phosphate-buffered saline (PBS) for 10 min on ice with frequent vigorous vortexing to

Table II. Fatty Acid Composition of Oils[a]

Fatty acid[b]	Percentage of total fatty acids		
	Corn oil (%)	Hydrogenated coconut oil (%)	Menhaden oil (%)
14:0	0.1	46.2	7.9
16:0	14.9	23.1	16.1
16:1	0	0	10.9
18:0	0	30.1	2.7
18:1	23.8	0	14.6
18:2	59.0	0	2.1
18:3	0	0	1.6
20:3	0	0	0.5
20:4	2.2	0	0
20:5	0	0	15.9
22:6	0	0	16.1

[a] Taken from Ref. 4.
[b] Only fatty acids comprising at least 0.5% of total fatty acids are shown.

produce a suspension of lipids at 2 mg/ml. Small unilamellar vesicles (SUVs) were produced by sonication with a Heat Systems sonicator (Level 5, 1-sec cycle) for 5 min in an ice bath. In some experiments, the SUV preparation was centrifuged at $145g_{av}$ to remove any particulate matter (from the sonicator) but this procedure did not alter the results observed. The SUVs (0.5 ml/mouse) were injected into the peritoneal cavity of tumor-bearing mice at the times shown in the figures. In one set of experiments, the tumor inoculum was mixed with the SUVs (liposomes) before injection.

Peroxidation Assay. Lipid peroxidation was measured with an assay for thiobarbituric acid (TBA) reactive substances. A malondialdehyde (MDA) stock was prepared by hydrolyzing malonaldehyde bis(diethylacetal) in 1% (v/v) sulfuric acid for 2 hr at room temperature (11). MDA molarity was determined from the absorbance at 245 nm ($\epsilon = 1.37 \times 10^4$) (12). A series of MDA standards ranging from approximately 0.5 to 15 nmoles was prepared in saline. Liposomes were tested in this assay in the same form in which they were administered to mice (2 mg/ml PBS). Ethanolic butylated hydroxytoluene was added to all standards and liposome samples at a final concentration of 20 μg/ml to prevent peroxidation during the assay (11). TBA (1 ml of 0.4% TBA in 0.2 N HCl) was added to each 0.5-ml standard and sample and the mixtures were heated in a boiling water bath for 15 min. After the tubes cooled, an equal volume of isobutanol was added; once the phases separated, fluorescence in the upper organic phase was measured in a Perkin Elmer MPF-66 fluorescence spectrophotometer at an excitation wavelength of 515 nm, emission wavelength of 550 nm, and 0.5-nm slit width. TBA-reactive substances in the liposome samples were quantified in terms of nmoles of MDA from a standard curve fitted by linear regression.

Flow Cytometry. Surface markers to characterize the T27A line were identified by incubating the T27A cells with monoclonal antibodies obtained from Becton Dickinson (Franklin Lakes, NJ)/(FITC-conjugated anti-Thy-1), Boehringer-Mannheim (H129.19, M17/5.2, 53.6.7; Indianapolis, IN) and ATCC hybridomas (SF1-1.1.1., 34-5-8S, 15-5-5S, M1/42.3.9.8, M17/4.4.11.9). Unlabeled primary antibodies were detected with FITC-conjugated goat anti-rat IgG and anti-mouse IgG (Kirkegaard and Perry, Gaithersburg, MD). FITC-conjugated goat anti-mouse IgM was purchased from Sigma Chemical Co. (St. Louis, MO). Background staining was measured with irrelevant primary antibodies and the FITC-conjugated secondary reagents. Standard analysis was performed on a Coulter EPICS Elite ESP cell sorter system equipped with an air-cooled argon laser.

Statistics. Analysis of variance (ANOVA) with a Newman-Keuls post hoc test and Kruskal-Wallis nonparametric ANOVA were used to assess differences in mouse age at death between treatment groups; P values ≤ 0.05 were accepted as significant.

Results and Discussion

Epidemiological and experimental data suggest that high-fat diets increase the incidence of certain cancers (13). Diets containing linoleic acid (e.g., corn oil) have been reported to enhance tumor growth, possibly by supplying that essential fatty acid (14, 15). In contrast, DHA-rich (i.e., fish oil–containing) diets reduce the growth and incidence of certain cancers and extend the lives of tumor-bearing animals (16–19), although this benefit is not unequivocal (20). In the current study, we have found that a menhaden (fish) oil–rich diet enhances the longevity of animals inoculated with even a very large (routinely fatal) T27A leukemia dose (Fig. 1). The excess tumor cell number was used to guarantee that enhanced host survival would not occur randomly due to tumor cell loss during manipulations; however, it is likely that smaller tumor doses would have accentuated the benefit of ω-3 fatty acids. In the experiment shown in Figure 1A, the mean and median survival times of fish oil–fed tumor-bearing mice were both 17.5 days, compared with 13.5 days for the hydrogenated coconut oil–fed mice ($P = 0.0047$, ANOVA). Similarly, in Figure 1B, fish oil–fed T27A tumor-bearing mice outlived corn oil–fed tumor hosts; the mean [median] survival times were 17 [16] days for the fish oil and 13 [13] days for corn oil–fed mice ($P = 0.0005$, ANOVA).

The mechanism of fish oil's benefit in extending longevity of the tumor-bearing host is not clear, because the ω-3 fatty acids in fish oil may have several nonexclusive actions. They reduce potent leukotriene and prostaglandin production (from arachidonic acid, 20:4ω6), are subject to peroxidation (and the produc-

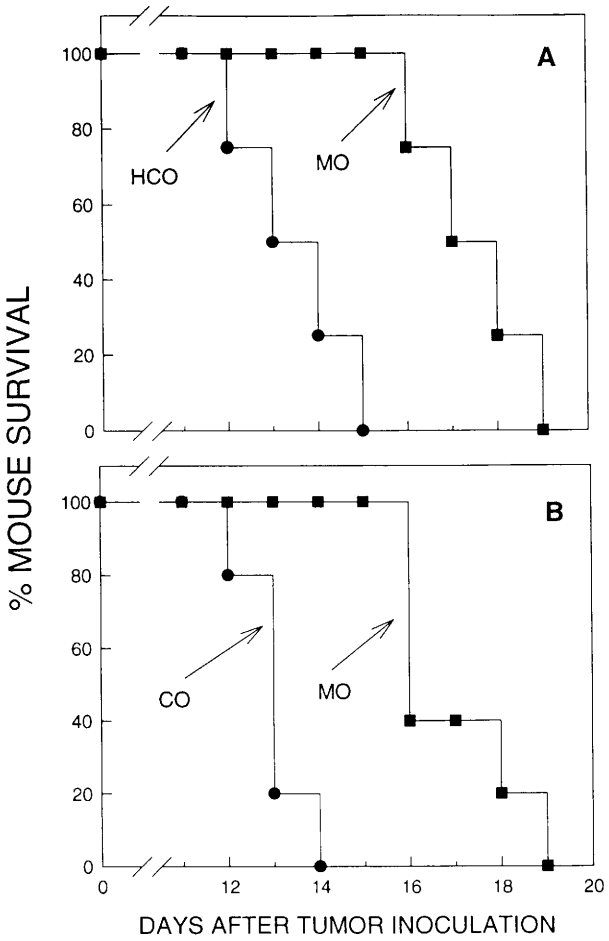

Figure 1. An ω-3 fatty acid–rich fish oil diet enhances longevity of tumor bearing-mice. BALB/c male mice were fed experimental diets for 1 week and then inoculated intraperitoneally with 1 × 10^4 T27A cells. Mouse survival was charted daily. (A) n = 4 mice per group; P = 0.0047, ANOVA. (B) n = 5 mice per group; P = 0.0005, ANOVA. CO, corn oil; HCO, hydrogenated coconut oil; MO, menhaden oil.

tion of free radicals), and in phospholipids alter the structure and presumably function of biological membranes. We reported previously that exposure of T27A cells to lipid vesicles of phosphatidylcholine with DHA in the *sn*-2 position (18:0, 22:6 PC) increased permeability (1) and altered surface protein expression (5), indicating a direct effect of DHA on plasma membrane structure and function. At very high concentrations, 18:0, 22:6 PC (but not oleic acid–containing phosphatidylcholine, 18:0, 18:1 PC) reduced cell viability (5). Thus, we predicted that, compared with other phospholipids, 18:0, 22:6 PC may inhibit tumor growth and viability and extend host longevity *in vivo*.

Our test of this prediction was divided into three sections: an attempt to rescue tumor-bearing animals by injection of SUVs (liposomes) during advanced stages of tumor growth, periodic injections during tumor growth, and concurrent administration of lipid vesicles and tumor cells. The control lipid was 18:0, 18:1 PC, which is abundantly represented in biological membranes, poorly fusogenic, and unlikely to significantly alter tumor cells. The first section, rescue of moribund animals, was clearly unsuccessful. All tumor-bearing animals died shortly thereafter, regardless of lipid vesicle administration (18:0, 18:1 PC and 18:0, 22:6 PC), and for humane reasons the experiment was not repeated. Instead, we tested whether liposomes administered earlier and more frequently during tumor development would show a significant benefit.

Because T27A is a very aggressive leukemia (Table I), mouse survival was tabulated every half day (light cycle and dark cycle). A normal mouse lifespan is ~1/30 that of humans, and therefore each additional day of life for the tumor-bearing mouse is theoretically equivalent to 1 month for a human. The data in Figure 2A demonstrate that 18:0, 22:6 PC liposome administration spaced every 48 hr (Day 1, 3, 5, 7, 9, and 11) enhanced median survival time by 2.5 days (P = 0.0052, ANOVA; n = 8 mice per group). When three injections were given on Day 1, 2, and 3 after inoculation with tumor (Fig. 2B), the 18:0, 22:6 PC–treated mice showed a median survival time 1 day longer than the 18:0, 18:1 PC-treated controls; 1 day in the natural history of this aggressive ascites tumor is the difference between an active mouse with a minimal tumor burden and a quiescent mouse with a clearly protuberant abdomen. However, the difference between the two treatment groups was not statistically significant (P = 0.0665, ANOVA; n = 5 mice per group). Failure to achieve statistical significance may be a reflection of small sample size, which we believe to be most likely, or an indication that the SUVs failed to contact the limited number of tumor cells present. When three liposome treatments were given on Day 3, 6, and 9 (Fig. 2C), 18:0, 22:6 PC significantly increased survival of the tumor-bearing hosts (P < 0.0001, ANOVA; n = 8 mice per group).

One should not directly compare time spans in different experiments (e.g., Fig. 2, A–C) because some variation in median survival times between experiments is to be expected. Variation between experiments is due, we believe, primarily to the particular ascites passage used, but there are also subtle differences between mouse litters and liposome preparations. However, significant differences between 18:0, 18:1 PC and 18:0, 22:6 PC treatments were routinely achieved. Thus, periodic exposure of tumor-bearing mice to purified 18:0, 22:6 PC SUVs enhanced their longevity by as much as 5 days (Fig. 2B), which we translate as equivalent to 5 months for humans.

Why is 18:0, 22:6 PC providing this advantage to the tumor-bearing host? The benefit is unlikely to be merely one of providing nutrients to retard wasting, since one treatment on Day 6 alone had no effect and the caloric content of the two lipids are similar. Furthermore, elicitation of an inflammatory response

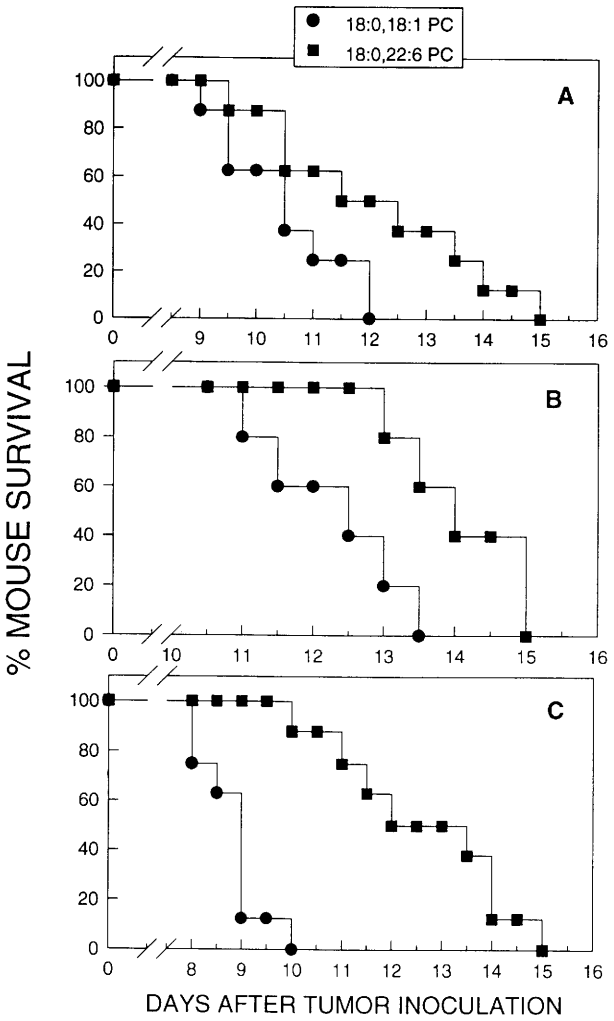

Figure 2. Administration of 18:0, 22:6 PC liposomes into the peritoneum of ascites tumor-bearing mice during tumor growth extends mouse longevity. T27A tumor cells (1×10^4 cells/mouse) were injected intraperitoneally on Day 0, and 0.5 ml of 18:0, 18:1 PC or 18:0, 22:6 PC liposomes (at a lipid concentration of 2 mg/ml of PBS) was injected into the peritoneum on various days thereafter. Mouse survival was tabulated by half days. (A) Liposomes were injected on Day 1, 3, 5, 7, 9, and 11 ($n = 8$ mice per group; $P = 0.0052$, ANOVA). (B) Liposomes were injected on Day 1, 2, and 3 ($n = 5$ mice per group; $P = 0.0665$, ANOVA). (C) Liposomes were injected on Day 3, 6, and 9 ($n = 8$ mice per group; $P < 0.0001$, ANOVA).

seems only a remote possibility. Arachidonic acid (20:4ω6) metabolites form potent inflammatory mediators (leukotrienes and prostaglandins), but administration of arachidonic acid–containing liposomes (18:0, 20:4 PC) did not result in significant enhancement of mouse longevity; 18:0, 20:4 PC treatment resulted in median survival time of 14.5 days, compared with 13.5 days for the 18:0, 18:1 PC–treated and 16.5 days for the 18:0, 22:6 PC–treated groups (Table III). In fact, the 18:0, 20:4 PC–treated group was not significantly different from the control (18:0, 18:1 PC) group in any of the three experiments in which 18:0, 20:4 PC was tested. α-Linolenic acid (18:3ω3) inhibits production of arachidonic acid metabolites, yet liposomes containing

Table III. Peroxidation Products Present in the Various Liposome Preparations

Liposomes	nmoles of MDA/mg lipid[a]	Median survival time[b] (days)
18:0, 18:0 PC	ND	11.0
18:0, 18:1ω9 PC	ND	13.5
18:0, 18:3ω3 PC	0.74 ± 0.71	18.0
18:0, 20:4ω6 PC	0.94 ± 0.64	14.5
18:0, 22:6ω3 PC	3.57 ± 0.17	16.5

[a] ND, not detected. Mean ± SD ($n = 3$)
[b] T27A tumor cells (1×10^4 cells per mouse) were injected ip, and the liposomes (0.5 ml at 2 mg/ml) were administered intraperitoneally on Day 3, 5, and 7. Mouse survival was charted ($n = 5$ mice per group).

α-linolenic acid (18:0, 18:3 PC) were effective at enhancing host survival, as were liposomes with the other ω-3 fatty acid DHA (18:0, 22:6 PC) (Table III). The sensitivity of the T27A line to eicosanoids has not been explored. It is possible that ω-3 fatty acids are inhibiting arachidonic acid metabolism and thereby inhibiting tumor growth (19). However, one would then also need to postulate that 18:0, 20:4 PC is itself reducing arachidonic acid metabolism.

Table III also indicates that peroxidation products are unlikely to mediate the liposomes' effect. The 18:0, 18:3 PC liposome preparation was beneficial, yet peroxidation was barely detectable. This is consistent with the observation that the rate of 18:3 peroxidation is much lower than DHA, which has twice the number of double bonds (21). 18:3 is a precursor in the biosynthetic pathway for 22:6; therefore, 22:6 levels *in vivo* may be elevated upon 18:3 administration. DHA-containing liposomes (18:0, 22:6 PC) had detectable peroxidation products (i.e., TBA-reactive substances), but these peroxidation products constituted a minute proportion of the total lipids (a maximum of 0.3%, assuming a 1:1 *M* ratio of MDA product to peroxidized lipid). This level of peroxidation seems unlikely to produce major structural changes in the tumor membranes exposed to the liposomes.

If liposomes affect tumor cells directly, then we would predict that administration of the tumor inoculum in the liposome suspension, which would maximize cell-lipid contact, would equal or exceed the efficacy of repeated liposome injections. The data in Figure 3 support this prediction. Concurrent administration of tumor and 18:0, 22:6 PC enhanced mouse survival, although statistical significance was achieved at the $P < 0.10$ but not 0.05 level ($P = 0.0929$, Kruskal-Wallis nonparametric ANOVA). Notably, one mouse receiving the tumor inoculum in 18:0, 22:6 PC survived tumor-free for 4 months after inoculation (Fig. 3). Our technical failure rate is so low (only two instances of failed tumor growth in ~14 years of weekly passage) that one is tempted to implicate the 18:0, 22:6 PC treatment in this mouse's disease-free

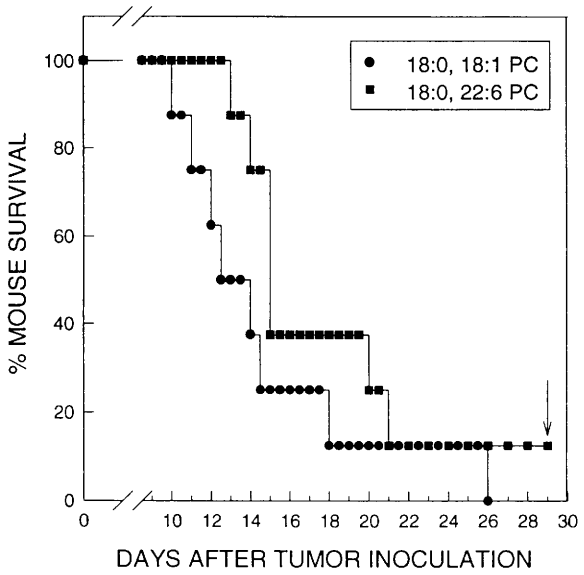

Figure 3. Concurrent administration of 18:0, 22:6 PC liposomes with tumor cells extends host longevity. Tumor cells were diluted in suspensions of 18:0, 18:1 PC or 18:0, 22:6 PC (each at 2 mg lipid/ml of PBS), and 0.5 ml of the lipid suspension containing 1×10^4 tumor cells was injected per mouse ($n = 8$ mice per group) on Day 0. No further liposome injections were made. Mouse survival was tabulated by half days. $P = 0.0929$, Kruskal-Wallis nonparametric ANOVA. (The 18:0, 22:6 PC–treated mouse indicated by the arrow survived 4 months disease-free.)

condition. In future studies, we wish to characterize ascites tumor cells harvested after liposome treatment, particularly with regard to tumor membrane structural changes directly attributable to the lipids. It should be noted that in these experiments, unlike those in Figure 2, the tumor cells were suspended in PBS (i.e., the liposome suspension), rather than the commercially formulated Hanks' balanced salt solution. Thus, some tumor cell death due to the austere PBS environment is likely, precluding direct comparison of Figure 2 and 3. Finally, although no undesirable side effects of repeated 18:0, 22:6 PC administration were noted that would preclude the use of large 18:0, 22:6 PC doses, the data in Figure 3 imply that massive 18:0, 22:6 PC amounts are not necessary to achieve a benefit. The total dose of lipid administered to the animals in Figure 3 was 1 mg (0.5 ml at 2 mg/ml), whereas in Figure 2B, for example, the total dose was 3 mg (0.5 ml at 2 mg/ml $\times$ 3 administrations).

Clearly, injection of 18:0, 22:6 PC at the site of tumor growth (i.e., the peritoneum of an ascites tumor-bearing host) is not a cure, but it offers a remarkable adjunct to other therapies. To our knowledge, 18:0, 22:6 PC liposomes have not been used as a drug delivery system, although both ω-3 and ω-6 polyunsaturated fatty acids in culture enhance tumor cell sensitivity to chemotherapeutic agents (22). There are some technical problems to be addressed, such as stabilizing the liposomal membrane and retarding clearance by the reticuloendothelial system (23). Liposome delivery of drugs is now commonplace; however, 18:0, 22:6 PC liposomes are normal components of biological systems that provide not only a delivery vehicle but also have inherent anticancer properties that are distinct mechanistically from those of the companion drug. Furthermore, the fusogenic properties of DHA greatly increase the likelihood that membrane impermeant drugs will be delivered to the tumor cells' interior. Without specific targeting, drugs encapsulated with 18:0, 22:6 PC could be delivered to local sites such as skin cancer and ovarian cancer metastases in the peritoneum.

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