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EFFECTS OF POLYUNSATURATED FATTY ACIDS ON THE EFFICACY OF ANTINEOPLASTIC AGENTS TOWARD L5178Y LYMPHOMA CELLS

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Abstract—Modification of cultured lymphoma cells (L5178Y) with individual unsaturated fatty acids [oleic acid (OA), linoleic acid (LA), α-linolenic acid (α-LNA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)] influenced cell growth and the responses of the cells to the chemotherapeutic agents doxorubicin (DRN), dexamethasone (DEX) and mitomycin-C (MTC). Cell proliferation generally decreased following modification with highly unsaturated fatty acids (>10 μM). The effects of drugs on growth varied with the type of fatty acid. Preincubation with α-LNA enhanced survival of L5178Y cells exposed to DRN. Modification with AA, EPA or DHA (>10 μM) reduced cell proliferation, particularly when cells were subsequently exposed to 50 or 100 nM DRN. There was no consistent relationship between fatty acid chain length, degree of unsaturation, and survival of cells when exposed to DEX or MTC. The data showed that modification of cultured L5178Y cells with highly unsaturated fatty acids, particularly DHA, enhances the toxic action of chemotherapeutic agents.

Manipulation of the sensitivity of actively dividing tumor cells to anti-cancer therapeutic agents is of importance in the control of cancer. Alteration of membrane lipids of tumor cells has the potential to alter membrane-related functions, e.g. responsiveness of membrane-bound receptors or expression of surface antigens, resulting in altered cellular function [1–4]. Tumor cells can synthesize fatty acids but readily utilize exogenous fatty acids for membrane phospholipid synthesis [4–6]. Growth and sensitivity of neoplastic cells to chemotherapeutic agents may be altered by the type of fatty acids incorporated [6–9].

Dietary lipids affect neoplastic growth *in vivo* and consumption of omega-6 fatty acids enhances growth of neoplasms [7–9]. Karmali *et al.* [9] reported that ingestion of omega-6 fatty acids (linoleic acid) increases the incidence in rats of dimethylbenzanthracene (DMBA†)-induced mammary tumors compared with rats consuming omega-3 polyunsaturated fatty acids. O'Connor *et al.* [7] observed that rats fed diets rich in omega-3 (n-3) fatty acids have reduced numbers of preneoplastic pancreatic lesions, following exposure to azaserine.

Murine leukemia cells (L1210) incubated with 40 μM docosahexaenoic acid (DHA) are 2.5 times more sensitive to hyperthermia (42°) exposure compared with tumor cells incubated with oleic acid (OA) [10]. Modifying the tumor cell membrane with DHA significantly enhances the sensitivity of L1210 murine leukemic cells to the antineoplastic agent doxorubicin (DRN) [5, 8–10]. The increase in sensitivity to DRN is related to the degree of unsaturation of fatty acyl chain [6].

This study was conducted to determine if the cytotoxic effects of the chemotherapeutic agents DRN, dexamethasone (DEX) and mitomycin-C (MTC) are changed following alteration of the lipid composition of target cells by preincubation with various unsaturated fatty acids. DRN is an anthracyclin antibiotic active against carcinomas and sarcomas [11]. DEX is used in the treatment of cancer cell lymphoma and melanoma [12], and MTC is an alkylating agent that interacts with DNA affecting cell proliferation [11].

In previous studies tumor cells were incubated with pharmacological concentrations of fatty acids (10^{-4} M), then exposed to a high concentration (μM) of the chemotherapeutic agent, and examined for cell death after a short time [13]. In the present study, we examined the effects of membrane modification with low levels of fatty acids on the effective toxicity of these agents at concentrations that closely approximated tissue concentrations observed *in vivo* following drug administration.

MATERIALS AND METHODS

Cell line. The continuous cell line utilized in this study was the methylcholanthrene-induced DBA/2 lymphoma, L5178Y cell line (obtained from Dr. E. F. Wheelock, Hahnemann University, Philadelphia, PA). These cells have been thoroughly characterized and used for metastatic studies [14, 15]. The cells were maintained as monolayers at 37° in a humidified 5% CO_2 atmosphere [14, 15], in Eagle's Minimum Essential Medium (MEM) containing 10% fetal calf

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† Abbreviations: AA, arachidonic acid; DEX, dexamethasone; DHA, docosahexaenoic acid; DMBA, dimethylbenzanthracene; DRN, doxorubicin; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; LA, linoleic acid; α-LNA, α-linolenic acid; MEM, Eagle's Minimum Essential Medium; MTC, mitomycin-C; OA, oleic acid; PUFAs, polyunsaturated fatty acids; and SA, stearic acid.

serum (FCS), L-glutamine (2 mM), sodium pyruvate (1 mM), 15 mM HEPES, non-essential amino acids (0.1 mM), sodium bicarbonate (1.125 mg/L), and gentamycin (50 μg/mL) (Hazleton Biologics, Lenexa, KS) in 75 cm^2 flasks (Corning Glass Works, Corning, NY). The cells were transferred every 3 days and new cultures were started from frozen stock every 10 passages as previously described [15].

The cells were harvested by rinsing nonadherent cells from the flasks and then adding 0.1% trypsin, 0.15 M EDTA in saline to cleave the adherent cells from the flask. The cells were rinsed three times and recovered by centrifugal sedimentation and resuspension in fresh MEM. Cell number was determined by a hemocytometer [15].

Fatty acids. Pure OA, linoleic acid (LA), linolenic acid (α-LNA), arachidonic acid (AA) and DHA were purchased from NuChek Prep (Elysian, MN). Eicosapentaenoic acid (EPA) was purchased from Cayman Chemical (Ann Arbor, MI). All fatty acids were greater than 99% pure as determined by gas chromatography. The fatty acids were diluted in ethanol and checked for oxidation by measuring absorbance at 230 nm. None of the fatty acid preparations had measurable peroxide formation (<0.1 nM). Known quantities of each of the fatty acids were added to medium containing 2.5% FCS for 24 hr before incubation with the cells to allow binding of fatty acids to the serum proteins [1, 13]. Negligible oxidation occurred during this period.

Drugs. DRN, DEX and MTC were purchased from the Sigma Chemical Co. (St. Louis, MO). Stock solutions and dilutions of DRN and MTC were prepared in sterile saline; DEX stock and dilutions were prepared in ethanol [11, 12].

Experimental procedure. In the first series of studies the L5178Y cells (2×10^5) were seeded into 36.4 mm × 6 well flat bottom plates (Corning Glass Works). The medium containing the fatty acid was added to each well to give a final fatty acid concentration of 0, 5, 10 or 25 μM (total volume, 2 mL/well) and incubated for 24 hr. Earlier studies showed that this was sufficient time to achieve maximum alteration of membrane phospholipids [6, 16]. Control cells were grown in medium without added fatty acids. After 24 hr, the drugs were added to the wells to a final drug concentration of 1, 10, or 100 nM and the cells were incubated in the presence of the drugs for 3 days to determine growth rate and survival of the L5178Y cells. The nonadherent cells were rinsed from the wells and the adherent cells were harvested by releasing from the plate with 0.1% trypsin/0.1 M EDTA (Hazleton Biologics) as described [17]. The cells were rinsed three times and immediately quantified by a Coulter counter [15].

The data are presented as percent survival relative to controls (incubated without exogenous fatty acids or drugs) using the formula:

$$\% \text{ Survival} = \frac{\text{cell number in treatment wells}}{\text{cell number in control wells}} \times 100$$

In the second series of studies, the cell culture conditions were the same as above; however, the exogenous fatty acid concentration was maintained at 10 μM and the drug concentrations used were 10, 50, and 100 nM. The cells were initially incubated with the fatty acids for 24 hr and then incubated for an additional 3 days after the addition of the drugs. Each treatment was done in quadruplicate. The data for cells incubated with the drugs were normalized to remove any effects attributable to the fatty acids by determining percent survival for each fatty acid using the formula:

$$\% \text{ Survival} = = \frac{\text{cell number with drug and with fatty acid}}{\text{cell number without drug but with the same fatty acid}} \times 100$$

Statistical analysis. Means, standard error, analysis of variance and Duncan's Multiple Range mean separation tests were calculated by the standard SAS statistics analysis system (SAS Institute, Cary, NC).

RESULTS

The L5178Y cells readily incorporated exogenous fatty acids into the membrane lipids, and the

Table 1. Effect of incubation with increasing concentrations of exogenous unsaturated fatty acids on the proliferation of L5178Y lymphoma cells*

Concn (μM)	Exogenous fatty acid					
	OA	LA	α-LNA	AA	EPA	DHA
	Cell number × 10^6					
0	2.45 ± 0.09^{a}	$^{ab}1.93 \pm 0.13^{b}$	$^{a}2.59 \pm 0.18^{a}$	$^{a}2.57 \pm 0.17^{a}$	$^{a}2.61 \pm 0.13^{a}$	$^{a}2.43 \pm 0.23^{a}$
5	2.49 ± 0.17^{ab}	$^{bc}2.18 \pm 0.11^{bc}$	$^{a}2.61 \pm 0.14^{a}$	$^{a}2.54 \pm 0.15^{ab}$	$^{a}2.65 \pm 0.11^{a}$	$^{a}1.82 \pm 0.06^{c}$
10	0.31 ± 0.13^{a}	$^{c}2.37 \pm 0.11^{a}$	$^{a}2.29 \pm 0.38^{a}$	$^{a}2.16 \pm 0.05^{a}$	$^{ab}2.13 \pm 0.20^{a}$	$^{a}1.81 \pm 0.07^{a}$
25	2.20 ± 0.15^{a}	$^{a}1.73 \pm 0.13^{ab}$	$^{b}1.45 \pm 0.09^{b}$	$^{b}1.24 \pm 0.16^{b}$	$^{b}1.64 \pm 0.29^{ab}$	$^{b}1.03 \pm 0.40^{b}$

Cells were grown with exogenous fatty acids for 4 days and were counted as described in the text. Abbreviations: OA, oleic acid; LA, linoleic acid; α-LNA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; and DH, docosahexaenoic acid.

* Values are means ± SEM, 3 wells/treatment. Means with different superscripts to the right of the values were significantly different ($P < 0.05$) for fatty acid type. Means with different superscripts to the left of the values were significantly different ($P < 0.05$) for fatty acid concentration.

Table 2. Effect of preincubation with increasing concentrations of unsaturated fatty acids on the survival of L5178Y lymphoma cells exposed to increasing concentrations of doxorubicin*

Concentrations		Exogenous fatty acid					
Drug (nM)	Fatty acid (μM)	OA	LA	α-LNA	AA	EPA	DHA
		Percent survival					
1	0	$^{a}101.4 \pm 7.9$	$^{a}101.4 \pm 7.9$	$^{a}101.4 \pm 7.9$	$^{a}101.4 \pm 7.9$	$^{b}101.4 \pm 7.9$	$^{a}101.4 \pm 7.9$
1	5	$^{a}111.7 \pm 5.5^{ab}$	$^{a}96.4 \pm 5.7^{cd}$	$^{a}116.6 \pm 3.3^{a}$	$^{a}99.3 \pm 0.8^{bc}$	$^{a}102.8 \pm 1.0^{abc}$	$^{ab}83.5 \pm 5.0^{d}$
1	10	$^{ab}90.6 \pm 5.7^{a}$	$^{a}89.2 \pm 1.8^{a}$	$^{a}107.7 \pm 3.3^{b}$	$^{a}70.3 \pm 8.4^{c}$	$^{a}102.1 \pm 1.1^{ab}$	$^{b}70.4 \pm 4.0^{c}$
1	25	$^{b}77.2 \pm 2.2^{a}$	$^{b}65.7 \pm 4.4^{ab}$	$^{b}57.3 \pm 6.7^{abc}$	$^{b}37.0 \pm 10.9^{c}$	$^{b}49.1 \pm 8.0^{bc}$	$^{c}13.2 \pm 1.8^{d}$
10	0	$^{a}102.6 \pm 2.9$	102.6 ± 2.9 ^{a}	102.6 ± 2.9	$^{a}102.6 \pm 2.9$	$^{a}102.6 \pm 2.9$	$^{a}102.6 \pm 2.9$
10	5	$^{a}99.0 \pm 1.5^{abc}$	92.2 ± 0.2^{bc}	$^{a}111.2 \pm 7.9^{a}$	$^{a}89.6 \pm 1.2^{c}$	$^{a}105.2 \pm 3.5^{ab}$	$^{bc}46.2 \pm 2.6^{d}$
10	10	$^{a}94.1 \pm 0.7^{ab}$	95.1 ± 2.7^{ab}	$^{a}118.5 \pm 1.4^{c}$	$^{a}75.4 \pm 13.4^{b}$	$^{a}99.1 \pm 0.6^{bc}$	$^{ab}77.3 \pm 7.4^{ab}$
10	25	$^{b}76.0 \pm 4.6^{ab}$	95.6 ± 4.0^{a}	$^{b}64.3 \pm 6.3^{bc}$	$^{b}41.2 \pm 3.3^{cd}$	$^{b}61.0 \pm 1.8^{bc}$	$^{c}31.2 \pm 15.9^{d}$
100	0	$^{a}32.2 \pm 2.5$	$^{a}32.2 \pm 2.5$	32.2 ± 2.5	$^{a}32.2 \pm 2.5$	32.2 ± 2.5	$^{a}32.2 \pm 2.5$
100	5	$^{b}50.7 \pm 5.2^{a}$	$^{b}69.2 \pm 0.9^{b}$	$^{a}32.6 \pm 1.2^{c}$	$^{b}70.0 \pm 3.7^{b}$	26.0 ± 1.6^{c}	$^{a}25.2 \pm 1.1^{c}$
100	10	$^{b}49.9 \pm 0.1^{a}$	$^{b}76.6 \pm 8.4^{b}$	$^{ab}23.3 \pm 3.3^{c}$	$^{c}45.4 \pm 2.2^{ad}$	25.5 ± 1.1^{c}	$^{a}29.4 \pm 8.2^{cd}$
100	25	$^{ab}46.2 \pm 4.3^{a}$	$^{a}20.0 \pm 1.0^{b}$	$^{b}20.4 \pm 3.0^{b}$	$^{d}11.8 \pm 0.5^{c}$	28.0 ± 1.6^{b}	$^{b}4.9 \pm 0.3^{c}$

* Cells were incubated in the presence of exogenous fatty acids for 1 day prior to addition of the drug. The cells were then incubated for an additional 3 days and counted as described in the text. See Table 1 for the definition of the abbreviations. Survival was determined as a percentage of cell number compared to control values with no added drug or fatty acid. Data are means ± SEM of quadruplicate treatments. Means with different superscripts to the left were significantly different ($P < 0.05$) for increasing fatty acid concentration within each drug concentration. Means with different superscripts to the right were significantly different ($P < 0.05$) for the type of fatty acid within each fatty acid concentration and drug concentration.

concentration of the exogenous fatty acid in the cellular phospholipids increased significantly with concentration added to the culture medium. LA was readily converted to AA by these cells. AA, EPA and DHA were avidly incorporated into cellular phospholipids (Kinsella, unpublished data).

The effects of the fatty acids on cell proliferation were related to the degree of unsaturation (Table 1). Cell proliferation was mostly affected by the polyunsaturated fatty acids (PUFAs) above 10 μM, especially by AA and DHA (Table 1).

The addition of drugs reduced cell proliferation and generally accentuated the effects of the highly unsaturated DHA above 10 μM (Tables 2–4).

Each of the drugs had differing potencies in reducing cell growth (Tables 2–4). MTC at 1 nM significantly ($P < 0.05$) reduced survival of L5178Y cells, and was the most potent of the three chemotherapeutic agents compared with DEX, which affected survival at 10 nM, while DRN at 100 nM ($P < 0.05$) reduced cell numbers.

The effects of increasing drug concentration on the growth of cells modified by increasing concentrations of specific fatty acids can be seen in the data (vertical columns) in Tables 2–4 while the effects of different species of fatty acids, at different levels and drug concentrations can be observed from the horizontal data rows (Tables 2–4).

Increasing concentrations of DRN progressively reduced cell multiplication in all cases, with this effect being accentuated with the extent of modification of cellular lipids, particularly with DHA, at all concentrations.

There was no significant reduction in cell proliferation when cells incubated with 5 μM fatty acid were exposed to any of the drugs. However, cells modified by DHA were susceptible when exposed to DRN or DEX (Tables 2 and 3). Compared with the other fatty acids, modification of cells with 5 μM LA or AA increased cell survival rates against DRN (Table 2).

Incubation with 10 μM fatty acids caused less inhibition than observed with 25 μM fatty acids. Proliferation of cells incubated with EPA was enhanced compared with AA or DHA when the cells were exposed to 1 or 10 nM DRN or DEX. However, the protective effect of EPA was not evident when cells were exposed to 100 nM DRN or DEX or any concentration of MTC (Tables 2–4).

A study was conducted to examine the effects of higher concentrations of drugs on cells modified by preincubation with 10 μM exogenous fatty acids, i.e. controlling for the different effects of fatty acids. The survival of L5178Y cells incubated with DRN was enhanced following preincubation with α-LNA compared with controls or cells modified with other fatty acids (Fig. 1). Increasing the chain length of fatty acids from 18 to 22 carbon fatty acids tended to reduce survival, particularly when the cells were incubated with 50 nM DRN. Modification with DHA consistently reduced survival of cells exposed to 50 and 100 nM DRN relative to cells modified with other fatty acids.

Modification of cells with OA or EPA increased survival compared with cells incubated with LA, α-LNA, or AA, following exposure to 10 or 100 nM DEX. Incubation of the cells with stearic acid (SA) tended to increase survival of cells exposed to 50 or 100 nM DEX. Incubation with DHA did not affect survival of the cells at any DEX concentration.

Incubation of the cells with α-LNA decreased survival most significantly when the cells were

Table 3. Effect of preincubation with increasing concentrations of unsaturated fatty acids on the survival of L5178Y lymphoma cells exposed to increasing concentrations of dexamethasone*

Concentrations		Exogenous fatty acid					
Drug (nM)	Fatty acid (μM)	OA	LA	α-LNA	AA	EPA	DHA
		Percent survival					
1	0	$^{ab}99.7 \pm 2.7$	$^{a}99.7 \pm 2.7$	$^{a}99.7 \pm 2.7$	$^{ab}99.7 \pm 2.7$	$^{a}99.7 \pm 2.7$	$^{a}99.7 \pm 2.7$
1	5	$^{c}112.2 \pm 2.0^{a}$	$^{ab}96.1 \pm 1.1^{b}$	$^{a}109.4 \pm 3.8^{a}$	$^{a}110.8 \pm 4.4^{a}$	$^{a}98.4 \pm 0.1^{b}$	$^{b}76.0 \pm 1.1^{c}$
1	10	$^{bc}103.8 \pm 1.6^{a}$	$^{ab}98.3 \pm 0.9^{b}$	$^{a}108.4 \pm 0.7^{c}$	$^{b}90.9 \pm 0.8^{d}$	$^{a}97.0 \pm 0.8^{c}$	$^{b}76.0 \pm 1.5^{e}$
1	25	$^{a}89.4 \pm 4.1^{a}$	$^{b}84.9 \pm 6.1^{ab}$	$^{b}64.8 \pm 12.0^{bc}$	$^{c}59.5 \pm 4.1^{cd}$	$^{b}82.3 \pm 5.4^{abc}$	$^{c}41.6 \pm 2.2^{d}$
10	0	79.0 ± 2.5	$^{a}79.0 \pm 2.5$	$^{a}79.0 \pm 2.5$	$^{a}79.0 \pm 2.5$	$^{ab}79.0 \pm 2.5$	$^{a}79.0 \pm 2.5$
10	5	80.7 ± 2.6^{a}	$^{a}77.8 \pm 0.5^{a}$	$^{a}92.3 \pm 3.4^{b}$	$^{a}76.4 \pm 3.9^{a}$	$^{a}82.8 \pm 1.8^{a}$	$^{b}65.4 \pm 0.1^{c}$
10	10	80.9 ± 5.8	$^{a}85.2 \pm 5.7$	$^{a}82.2 \pm 9.7$	$^{a}79.4 \pm 0.5$	$^{a}82.4 \pm 4.7$	$^{b}68.4 \pm 2.4$
10	25	84.9 ± 0.1^{a}	$^{b}62.3 \pm 0.1^{b}$	$^{b}45.1 \pm 2.2^{c}$	$^{b}44.0 \pm 4.9^{c}$	$^{b}70.5 \pm 0.6^{d}$	$^{c}29.8 \pm 1.3^{e}$
100	0	$^{a}46.8 \pm 0.8$	$^{a}46.8 \pm 0.8$	$^{a}46.8 \pm 0.8$	$^{a}46.8 \pm 0.8$	$^{a}46.8 \pm 0.8$	$^{a}46.8 \pm 0.8$
100	5	$^{ab}59.5 \pm 4.7^{a}$	$^{b}62.6 \pm 0.7^{ab}$	$^{b}72.1 \pm 1.4^{b}$	$^{b}64.2 \pm 3.9^{ab}$	$^{b}65.6 \pm 2.4^{ab}$	$^{b}62.1 \pm 3.3^{ab}$
100	10	$^{b}62.1 \pm 3.2^{ab}$	$^{b}61.1 \pm 1.9^{ab}$	$^{c}63.1 \pm 0.7^{ab}$	$^{b}65.1 \pm 0.1^{a}$	$^{b}60.4 \pm 3.3^{ab}$	$^{ab}56.8 \pm 1.3^{b}$
100	25	$^{ab}54.1 \pm 3.5^{a}$	$^{a}46.0 \pm 6.2^{ab}$	$^{a}45.2 \pm 0.4^{ab}$	$^{c}33.2 \pm 2.3^{bc}$	$^{ab}54.7 \pm 3.8^{a}$	$^{c}25.7 \pm 3.7^{c}$

* Cells were incubated in the presence of fatty acids and drugs and counted, as described in the text. See Table 1 for the definition of the abbreviations. Survival was determined as a percentage of cell number compared to control values with no added drug or fatty acid. Data are means ± SEM of quadruplicate treatments. Means with different superscripts to the left were significantly different ($P < 0.05$) for increasing fatty acid concentration within each drug concentration. Means with different superscripts to the right were significantly different ($P < 0.05$) for the type of fatty acid within each fatty acid concentration and drug concentration.

Table 4. Effect of preincubation with increasing concentrations of unsaturated fatty acids on the survival of L5178Y lymphoma cells exposed to increasing concentrations of mitomycin-C*

Concentrations		Exogenous fatty acid					
Drug (nM)	Fatty acid (μM)	OA	LA	α-LNA	AA	EPA	DHA
		Percent survival					
1	0	$^{a}105.7 \pm 1.3$	$^{a}105.7 \pm 1.3$	$^{a}105.7 \pm 1.3$	$^{a}105.7 \pm 1.3$	$^{a}105.7 \pm 1.3$	$^{a}105.7 \pm 1.3$
1	5	$^{b}90.3 \pm 0.8^{a}$	$^{b}124.2 \pm 0.5^{b}$	$^{a}98.4 \pm 2.9^{b}$	$^{a}109.5 \pm 12.2^{ab}$	$^{ab}100.9 \pm 3.7^{b}$	$^{a}95.9 \pm 4.1^{b}$
1	10	$^{b}87.8 \pm 5.0^{ab}$	$^{a}102.2 \pm 1.0^{c}$	$^{b}46.8 \pm 0.3^{c}$	$^{a}102.4 \pm 0.5^{c}$	$^{b}90.0 \pm 4.3^{a}$	$^{b}78.6 \pm 0.1^{b}$
1	25	$^{a}108.0 \pm 1.4^{a}$	$^{c}66.1 \pm 6.2^{b}$	$^{b}51.9 \pm 2.7^{cd}$	$^{b}57.4 \pm 3.6^{bc}$	$^{c}43.6 \pm 1.6^{d}$	$^{c}27.8 \pm 3.8^{e}$
10	0	83.5 ± 2.1	$^{a}83.5 \pm 2.1$	$^{a}83.5 \pm 2.1$	$^{a}83.5 \pm 2.1$	$^{a}83.5 \pm 2.1$	$^{a}83.5 \pm 2.1$
10	5	82.2 ± 2.4^{ab}	$^{b}107.0 \pm 9.2^{c}$	$^{a}81.7 \pm 3.5^{ab}$	$^{b}96.8 \pm 0.1^{bc}$	$^{ab}79.4 \pm 0.6^{a}$	$^{a}78.6 \pm 2.3^{a}$
10	10	81.2 ± 3.7^{ab}	$^{ab}87.8 \pm 4.1^{a}$	$^{a}88.6 \pm 3.3^{a}$	$^{c}75.4 \pm 0.5^{b}$	$^{b}73.0 \pm 1.7^{b}$	$^{a}73.6 \pm 3.0^{b}$
10	25	82.4 ± 4.9^{a}	$^{c}51.5 \pm 2.9^{b}$	$^{b}45.4 \pm 2.0^{bc}$	$^{d}45.0 \pm 2.0^{bc}$	$^{c}37.4 \pm 2.9^{c}$	$^{b}21.9 \pm 3.1^{d}$
100	0	18.4 ± 0.1	$^{a}18.4 \pm 0.1$	$^{a}18.4 \pm 0.1$	$^{a}18.4 \pm 0.1$	$^{a}18.4 \pm 0.1$	$^{a}18.4 \pm 0.1$
100	5	22.2 ± 2.7^{ab}	$^{b}26.1 \pm 0.5^{a}$	$^{a}18.8 \pm 0.4^{b}$	$^{a}17.9 \pm 1.9^{b}$	$^{a}18.5 \pm 0.2^{b}$	$^{a}18.5 \pm 1.9^{b}$
100	10	19.3 ± 1.6^{a}	$^{a}19.2 \pm 0.3^{a}$	$^{b}23.3 \pm 0.5^{b}$	$^{a}16.6 \pm 0.6^{a}$	$^{a}18.3 \pm 0.2^{a}$	$^{a}19.1 \pm 0.5^{a}$
100	25	16.5 ± 3.0^{a}	$^{c}13.4 \pm 0.8^{a}$	$^{c}14.2 \pm 1.6^{a}$	$^{b}11.3 \pm 0.1^{ab}$	$^{b}12.9 \pm 0.7^{ab}$	$^{b}7.7 \pm 0.9^{b}$

* Cells were incubated in the presence of fatty acids and drugs, and counted, as described in the text. See Table 1 for the definition of the abbreviations. Survival was determined as a percentage of cell number compared to control values with no added drug or fatty acid. Data are means ± SEM of quadruplicate treatments. Means with different superscripts to the left were significantly different ($P < 0.05$) for increasing fatty acid concentration within each drug concentration. Means with different superscripts to the right were significantly different ($P < 0.05$) for type of fatty acid within each fatty acid concentration and drug concentration.

exposed to MTC (Fig. 1c). In the presence of 100 nM MTC, cells modified with OA, LA and α-LNA showed significantly lower proliferation compared with controls. Preincubation with SA or AA tended to enhance survival relative to cells modified with OA, LA, α-LNA, EPA, or DHA.

DISCUSSION

Incubation of L5178Y cells with various fatty acids affected proliferation. The highly unsaturated DHA, in particular, depressed cell number at all concentrations. At 25 μM the unsaturated fatty acids

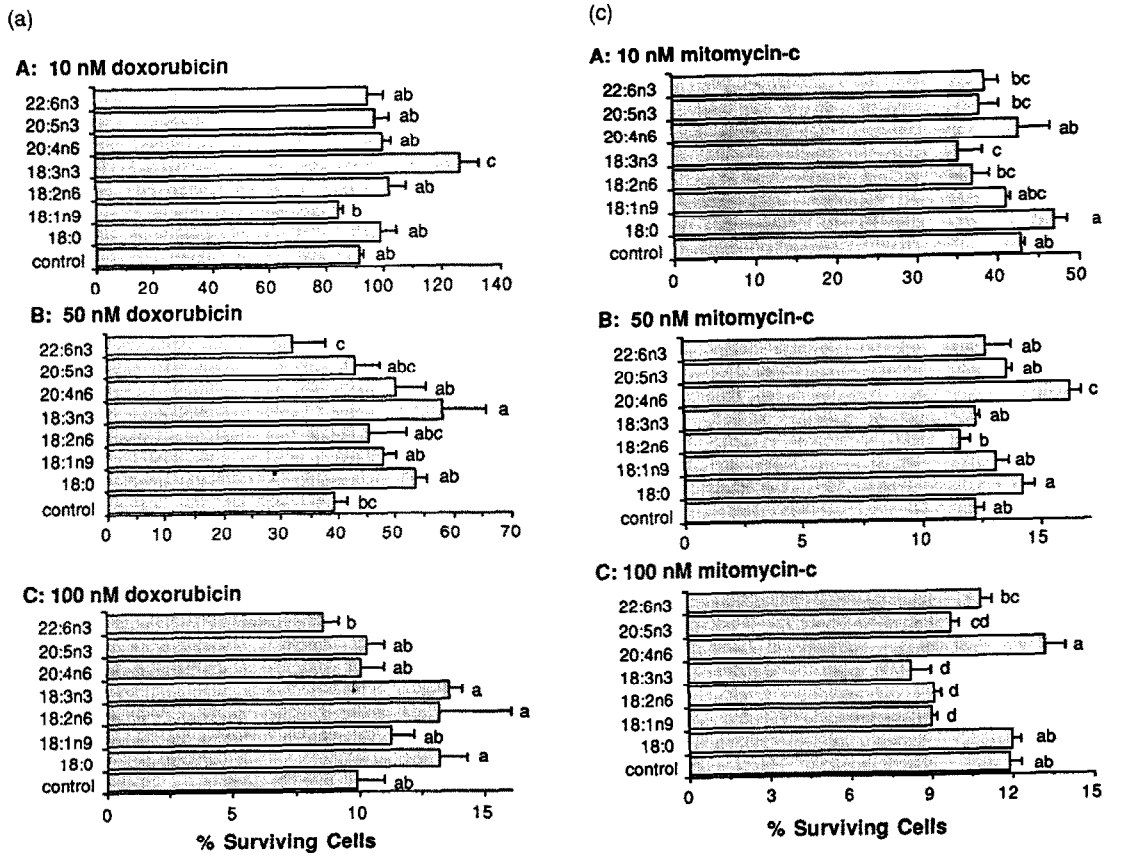

Fig. 1. Effect of preincubation with different exogenous fatty acids on the cytotoxicity toward L5178Y lymphoma cells exposed to increasing concentrations of three drugs: (a) doxorubicin; (b) dexamethasone; and (c) mitomycin-C. Cells (2×10^5) were seeded and preincubated with a 10 μM concentration of exogenous fatty acids. After 24 hr the drug was added to the wells for a final drug concentration of 10, 50 or 100 nM. The cells were then incubated for 3 days. Results are presented as percent surviving cells relative to cells incubated with exogenous fatty acids but without added drug. Data are means ± SEM of quadruplicate treatments. Bars with different letters were significantly different within a drug concentration ($P < 0.05$). Key: 22:6n3, docosahexaenoic acid (DHA); 20:5n3, eicosapentaenoic acid (EPA); 20:4n6, arachidonic acid (AA); 18:3n3, α-linolenic acid (α-LNA); 18:2n6, linoleic acid (LA); 18:1n9, oleic acid (OA); and 18:0, stearic acid (SA).

reduced cell numbers by at least 10% relative to controls, and this effect generally increased with increasing chain length and degree of unsaturation. This observation is of interest because the normal concentrations of free fatty acids in serum are in the range of 0.3 to 1.2 mM [16], which is at least 10-fold higher than the level of exogenous fatty acid used in this study. This suggests that *in vivo* cells may be susceptible to cytotoxic action following modification of membrane fatty acids via dietary manipulation.

Unsaturated fatty acids can have a direct cytotoxic action on tumor cells *in vitro* and this cytotoxic effect increases with increasing unsaturation and chain length [18, 19]. Siegel and coworkers [18] reported that 40 μM AA is the minimum concentration required to kill ascites tumor cells compared with 175 μM OA when the cells are incubated with the fatty acids for 7–9 days. The exact mechanism by which unsaturated fatty acids cause cell death is unknown; however, cells may be killed by a nonspecific detergent effect of the fatty acids on the cell membrane [20]. Another possible mechanism is that incorporation of highly unsaturated fatty acids into the phospholipids increases the potential for peroxidative damage [21].

The data from this study corroborate an apparent relationship between extent of unsaturation of fatty acids and cell death when tumor cells are exposed to chemotherapeutic agents [6]; however, there were exceptions. Survival of cells incubated with EPA and subsequently exposed to DRN or DEX was higher than that of cells incubated with AA or DHA. Incubation with OA gave a protective effect when L5178Y cells were exposed to DRN and did not affect cell proliferation relative to controls when exposed to DEX or MTC. However, increasing DHA content generally decreased survival at all drug concentrations. These data are consistent with those of Guffy *et al.* [8] who reported enhanced cytotoxicity of DRN to cells modified by DHA. Incorporation of DHA has a membrane fluidizing effect [10] that influences permeability, activity of membrane-associated enzymes [22], and transport systems [4]. DRN may independently increase membrane fluidity [23]. Hence, long-chain PUFAs may act synergistically with DRN in increasing membrane fluidity. It is known that DRN can be actively transported into the cells where it exerts its main effect on DNA synthesis [24]. However, DRN may also promote oxidation of cell membrane lipids [11]. Incorporation of highly unsaturated fatty acids such as DHA could predispose the cell to greater oxidative damage by DRN.

There are no reports concerning the effects of preincubation with fatty acids on the efficacy of DEX or MTC; however, it is known that DEX is readily bound to and may be transported by membrane receptors [12], which may be affected by the degree of unsaturation of membrane fatty acids [3].

The mechanism for the protective effect of EPA on the survival of the cells, exposed to DRN or DEX is not clear. This protective effect was not observed when cells were exposed to MTC, which may indicate that EPA reduces active transport of DRN and DEX into the cells. Exogenous AA, EPA and DHA are all readily incorporated into the phospholipid classes of L5178Y cells [17], therefore, this difference cannot be explained by differential incorporation into the phospholipids. However, EPA may be incorporated into different species of the phospholipid classes compared with AA or DHA, which may cause a lessened effect relative to AA or DHA [17].

In summary, incubation of L5178Y lymphoma cells with unsaturated fatty acids reduced cell proliferation and this reduction was enhanced when cells were exposed to chemotherapeutic agents. Survival of L5178Y cells was generally reduced with increasing unsaturation and chain length of the exogenous fatty acids. The effect of long-chain PUFAs may be attributed to predisposing the cell to injury, i.e. by increasing oxidation potential and membrane permeability that may alter membrane-bound enzyme function and transport. Modification with highly unsaturated fatty acids, particularly DHA, in combination with antineoplastic agents, which function by damaging cellular function such as replication and protein synthesis, may improve the potency of chemotherapeutic agents.

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