Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phosphatase-mediated process

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Abstract

Docosahexaenoic acid (DHA) is an omega-3 fatty acid under intense investigation for its ability to modulate cancer cell growth and survival. This research was performed to study the cellular and molecular effects of DHA. Our experiments indicated that the treatment of Jurkat cells with DHA inhibited their survival, whereas similar concentrations (60 and 90 µM) of arachidonic acid and oleic acid had little effect. To explore the mechanism of inhibition, we used several measures of apoptosis to determine whether this process was involved in DHA-induced cell death in Jurkat cells. Caspase-3, an important cytosolic downstream regulator of apoptosis, is activated by death signals through proteolytic cleavage. Incubation of Jurkat cells with 60 and 90 µM DHA caused proteolysis of caspase-3 within 48 and 24 h, respectively. DHA treatment also caused the degradation of poly-ADP-ribose polymerase and DNA fragmentation as assayed by flow cytometric TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay. These results indicate that DHA induces apoptosis in Jurkat leukemic cells. DHA-induced apoptosis was effectively inhibited by tautomycin and cypermethrin at concentrations that affect protein phosphatase 1 (PP1) and protein phosphatase 2B (PP2B) activities, respectively, implying a role for these phosphatases in the apoptotic pathway. Okadaic acid, an inhibitor of protein phosphatase 2A, had no effect on DHA-induced apoptosis. These results suggest that one mechanism through which DHA may control cancer cell growth is through apoptosis involving PP1/PP2B protein phosphatase activities. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Docosahexaenoic acid; Jurkat cell; Apoptosis; Caspase-3; Protein phosphatase

1. Introduction

The healing properties of fish oil have long been acknowledged as beneficial to the human body. The wide ranging effects of this dietary nutrient have been shown to play instrumental roles in reducing cardiac ailments such as atherosclerosis, total cardiac arrhythmia, and coronary heart disease; promoting normal neurological and ocular development; and repressing the evolution of cancer [1–5]. Epidemiology studies have associated a lower incidence of
cancer with dietary fish oil [6-8]. Much research has attributed all of these beneficial effects to fish oil's high content of omega-3 fatty acids, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

Many anticancer drugs exert their influence by inducing apoptosis. Apoptosis, or programmed cell death, is the physiological method through which unwanted or unneeded cells are eliminated during development or other biological processes [9]. It is also an important process in degenerative diseases, autoimmune disorders, and neoplasia development [10]. As a genetically regulated mechanism, apoptosis can occur through many pathways, but is classified by several typical cellular and molecular events such as cell shrinkage, dilation of endoplasmic reticulum, membrane blebbing, and extensive nuclear fragmentation [9]. Caspases, a family of cysteine proteases, play a critical role in apoptosis and are responsible for many of the biochemical and morphological changes associated with apoptosis [11-14].

It is interesting that DHA prevents apoptosis in retinal and neuronal cell lines [15,16]. DHA also protects neuro2A cells from serum-deprivation-induced apoptosis [15] and prevents spontaneous activation of the apoptotic pathway in retina photoreceptor cells grown in vitro [16]. DHA is reported to reduce sphingosine-induced apoptosis of the myeloid leukemia line HL-60 [17]. In sharp contrast, several other reports have demonstrated that DHA and EPA induce apoptosis of various normal and cancer cell lines [18-21]. Dietary supplementation with DHA (as a pure agent or in fish oil) increased apoptotic cell death in normal rat colonic cells [22-26], and the transplantable rat Morris hepatocarcinoma 3924A [27], exerted suppressive effects on the progression of human breast cancer cells MDA-MB-231 in athymic nude mice [28,29], increased survival time for dogs with lymphoma [30], reduced risk of prostate cancer in humans [31], and significantly reduced the incidence of tumor induction by dimethylbenz[a]anthracene in rats [32]. Addition of DHA to cultures of lung carcinoma A427 [33], Hep2 human larynx tumor cells [34], pancreatic Mia-Pa-Ca-2 cells [35], and embryonal carcinoma Tera-2 cells [36] induced apoptosis in these cell lines. DHA also inhibited growth of cervical cells immortalized by highly oncogenic human papillomavirus 16 (HPV16), foreskin keratinocytes immortalized by HPV16, and keratinocytes grown from papillomas with an HPV etiology [37]. Furthermore, conjugated DHA with a triene structure has been shown to induce apoptosis in DLD-1 cells (colorectal adenocarcinoma) without any effect on normal human fibroblast cell lines [38]. At present, the cellular and molecular mechanisms of DHA-induced apoptosis are unclear, and a better understanding of basic actions of DHA is needed before this polyunsaturated fatty acid can be fully employed in the clinic as an anticancer agent [39,40].

Several studies have shown that changes associated with apoptosis are mediated through activation of protein phosphatases [41-47]. Wolf and Eastman [44] have demonstrated that activation of protein phosphatase 1 (PP1) plays an important role in Fas-induced apoptosis by stimulating mitochondrial release of cytochrome c and caspase activation in HL-60 and Jurkat cells. Similarly, activation of a PP2A-like phosphatase has been demonstrated to play a key role in the induction of apoptosis in a neuronal cell line [43]. Several other studies have shown that a ceramide activated-protein phosphatase (CAPP), which is a member of protein phosphatase 2A, is involved in receptor-mediated induction of apoptosis in various cell lines [45]. These studies suggest that protein phosphatase activation may be a common feature of cells undergoing apoptosis. The present study was therefore undertaken to explore the role of protein phosphatases in DHA-induced apoptosis in Jurkat leukemic cell lines.

2. Materials and methods

2.1. Materials

The Jurkat cell line (Clone E6-1) was obtained from ATCC (American Type Culture Collection, Rockville, MD). RPMI 1640 was purchased from BioWhittaker (Walkersville, MD). Bovine calf serum (BCS) containing 3.6 g% albumin came from HyClone Laboratories (Logan, UT). Docosahexaenoic acid and arachidonic acid were obtained from NuChek-Prep, (Elysian, MN). Monoclonal anti-caspase-3 antibody was obtained from Transduction Laboratories (Lexington, KY). The polyclonal anti-PARP antibody and TUNEL flow cytometry kit were ob-
tained from Roche/Boehringer Molecular Biochemicals (Indianapolis, IN). The peroxidase-conjugated anti-mouse and anti-rabbit antibodies, the ECL kit, hyperfilm-HP, and the hyperfilm-ECL X-ray films came from Amersham Pharmacia Biotech (Buckinghamshire, UK). BCA Protein Assay kit came from Pierce (Rockford, IL). The pre-cast 12.5% SDS-PAGE gels were acquired from Owl Separation Systems (Portsmouth, NH). The polyvinylidene difluoride (PVDF) membranes were supplied from Millipore (Bedford, MA). Okadaic acid, tautomycin, and cypermethrin came from Calbiochem-Novabiochem, San Diego, CA. Permethrin and caspase-3 inhibitor DEVD-CHO (N-acetyl-Ala-Ala-Val-Ala-Leu-Leu-Pro-Ala-Val-Leu-Ala-Leu-Ala-Pro-Asp-Glu-Val-Asp-CHO, aldehyde) came from Biomol Research Laboratories (Plymouth Meeting, PA). Oleic acid and all chemicals and reagents used to prepare buffers and solutions herein mentioned were supplied by Sigma (St. Louis, MO).

2.2. Cell culture

The Jurkat T-cell leukemia cells were grown in RPMI 1640, supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin, 2 mM glutamine, and 2% bovine calf serum. The cells were kept in a humidified atmosphere of 37°C and 5% CO₂. Cell viability was determined by trypan blue exclusion. The Jurkat cells were treated with various concentrations of DHA, AA, or OA. DHA and AA were dissolved in ethanol, so that the final concentration of ethanol in culture medium did not exceed 0.05%. Oleic acid was suspended into medium prior to use. All fatty acid stocks were stored in small aliquots at −70°C under nitrogen, as described, to prevent their degradation and/or oxidation [48]. The working solutions of fatty acids were freshly prepared each time prior to their use.

2.3. Western blotting

After incubation, the cells were lysed with 50 μl of lysis buffer containing 20 mM Tris–HCl (pH 8), 137 mM NaCl, 100 mM NaF, 2 mM Na₃VO₄, 10% v/v glycerol, 1% v/v Nonidet P-40, 2 mM PMSF, 1 mg/ml leupeptin, 0.15 units/ml aprotinin, and 2.5 mM DIFP for 10 min on ice. Protein concentrations in the cell lysate were measured with a BCA protein assay system (Pierce), and equal amounts of detergent-solubilized protein extracts were resolved on 12.5% SDS–polyacrylamide gels as described previously [49]. The resolved proteins were electrophoretically transferred onto a PVDF membrane. Residual binding sites on the membranes were blocked with TTBS (50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05% Tween-20) containing 2% bovine serum albumin (BSA). The blots were then incubated with primary antibody (anti-caspase-3 or anti-PARP, 1:1000) in 1% BSA in TTBS for 1 h at room temperature. The membranes were then washed with TTBS and incubated with peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:1000 dilution in 1% BSA in TTBS). Blots were finally developed using an enhanced-chemiluminescence (ECL) kit and ECL x-ray film.

2.4. TUNEL flow cytometry assay

TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay was performed by using an In Situ Cell Death Detection kit (Roche/Boehringer Molecular Biochemicals) as recommended by the manufacturer. Briefly, after incubation with DHA, the cells (1 × 10⁵/100 μl) were fixed by adding 200 μl of 1% paraformaldehyde solution and incubated for 30 min at room temperature. These cells were washed once in phosphate-buffered saline (PBS) and then suspended in 100 μl of permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 min on ice. The cells were then washed twice with PBS and incubated with the TUNEL reaction mixture, as supplied by the manufacturer, in a humidified, dark, 37°C atmosphere for 1 h. The cells were then washed and analyzed using a Becton–Dickinson FACStarPLUS flow cytometer (San Jose, CA) equipped with a 488-nm wavelength argon laser operated at 200 mW. The fluorescein was detected through a 530 ± 20 band-pass filter.

2.5. Protein labeling with [³²P]ortho-phosphate

Jurkat cells (3 × 10⁶/ml) were washed twice with serum and phosphate-free RPMI (Gibco, Life Technologies) and incubated with [³²P]ortho-phosphate (100 μCi/ml) for 3 h. Cells were washed with
RPMI containing 2% BCS 3 times to remove unincorporated label. The radiolabeled cells (1×10^5/ml) were then incubated with 60 μM DHA. After each incubation time, cells were lysed with 50 μl lysis buffer, their protein contents were determined, and equal amounts of protein from each sample were resolved on a 7.5% SDS–polyacrylamide gel as described above. The resolved proteins were transferred on a PVDF membrane and then stained with a reversible Amido Black stain (Sigma) to determine the pattern of the resolved proteins. The same blot was then subjected to autoradiography using Amersham Hyperfilm MP for overnight exposure to determine the pattern of phosphorylated/dephosphorylated proteins.

3. Results

3.1. Effect of fatty acids on survival of Jurkat leukemic cells

Cells cultured in the presence of various concentrations of fatty acids (0–90 μM) for incubation times ranging from 0 to 48 h showed different viability trends depending on which fatty acid was used. As shown in Fig. 1a, low concentrations of DHA (30 μM) had no effect on cell viability; however, higher concentrations (60 and 90 μM) for extended periods of incubation time (24 and 48 h) markedly decreased the viability of the cells. The lowest cell viability, 28%, was produced by 90 μM DHA after 48 h of incubation. In contrast to these results, AA had little effect on Jurkat cell viability (Fig. 1b). The maximum concentration of AA (90 μM) with the longest incubation time (48 h) reduced Jurkat cell viability by only 20% compared to the control cells. As illustrated in Fig. 1c, OA also did not substantially affect Jurkat cell survival. These results clearly demonstrated that destruction of Jurkat leukemic cells is enhanced by DHA compared to AA and OA. In subsequent experiments, we studied whether the destruction of Jurkat cells in the presence of DHA is due to induction of apoptosis.

Fig. 1. Effects of oleic acid, arachidonic acid, and docosahexaenoic acid on cell viability. Jurkat cells (1×10^5/ml) were incubated in the presence of (●) 0 μM, (▼) 30 μM, (■) 60 μM, and (▲) 90 μM concentrations of (a) docosahexaenoic acid, (b) arachidonic acid, or (c) oleic acid for different time periods in a 37°C incubator as described in the text. Control cells were incubated with similar amounts of solvent used to dissolve these fatty acids. After each incubation time, cell viability was assayed by determining the trypan blue dye exclusion. Results represent mean ± S.E.M. for the 3 experiments.
3.2. DHA treatment of Jurkat cells induces caspase-3 activation

Caspase-3 belongs to a family of cysteine proteases that are involved downstream in the process of apoptosis. Caspases in cells are present as inactive proenzymes, which undergo proteolytic cleavage to become active enzymes. Fig. 2 demonstrates a DHA dose- and time-dependent induction of caspase-3 activation in Jurkat cells, i.e., the intensity of the caspase-3 band seen at 35 kDa is decreased and that of the 18 kDa processed form increased as the DHA concentration and incubation time increased. This finding was further confirmed when cells were preincubated with a highly specific and cell permeable caspase-3 inhibitor. The results presented in Fig. 3 demonstrate that pre-treatment of cells with caspase-3 inhibitor significantly (P < 0.001, Student’s t-test) improved the viability of cells from 61% (untreated cells) to 88% (treated cells). These findings suggest that DHA may affect the survival of Jurkat cells by inducing events upstream, leading to the activation of caspase-3, and ultimately leading to cell death.

3.3. DHA treatment induces PARP proteolysis in Jurkat cells

Because of the notable relation between DHA-induced cell death and caspase-3 activation, we further explored the effect of DHA on indicators of apoptosis. One of the direct substrates of caspase-3 is poly-
adenosine diphosphate-ribose polymerase (PARP), which is cleaved during apoptosis from 113 kDa to 89 and 24 kDa fragments. The results shown in Fig. 4 demonstrate the cleavage of PARP upon exposure to DHA. The 89 kDa subunit is apparent within 24 h of incubation in 90 µM DHA and by 48 h of incubation in 60 µM DHA. The 24 kDa fragment of PARP proteolysis was not detected by the anti-PARP antibodies. These data imply that activation of caspase-3 by DHA in Jurkat cells results in degradation of PARP enzymes.

3.4. DHA-Treated cells show DNA fragmentation as reported by TUNEL assay

The hypothesis that DHA induces apoptosis in Jurkat cells was further investigated by testing one of the hallmarks of apoptosis, DNA fragmentation. DHA-induced DNA fragmentation was measured on an individual cell basis using flow cytometry; fluorescence intensity is directly proportional to the degraded DNA present within each cell. The upper panel of Fig. 5 provides the raw flow cytometric data and also shows the gates established to calculate the percent of cells undergoing apoptosis (i.e., with fluorescence above the negative control). The lower panel of Fig. 5 shows summarized data illustrating the DHA dose-dependent increase in DNA fragmentation (mean fluorescent intensity) apparent after 48 h of DHA exposure. These results suggest that approximately 70% of the Jurkat cells treated with 90 µM DHA for 48 h undergo apoptosis.

3.5. DHA-Induced cell destruction is mediated through protein phosphatase activation

To explore our hypothesis that protein phosphatases are involved in DHA-induced apoptosis, we incubated Jurkat cells in the presence of various serine/threonine phosphatase (PP1, PP2A, PP2B) inhibitors during DHA-induced apoptosis. Results shown in Fig. 6 indicate that 10 nM okadaic acid (OKA), a concentration known to inhibit PP2A, did not significantly improve cell viability during 24 or 48 h of incubation with DHA. In contrast, 10 nM tautomycin (TMY) or 500 pM cypermethrin (CMT) in concentrations that effectively block PP1 or PP2B, respectively, reduced DHA-induced destruction of Jurkat cells (P < 0.001, Student’s t-test). The role of protein phosphatases PP1 and PP2B in the prevention of DHA-induced cell destruction was further analyzed by using other active and inactive analogues of protein phosphatase inhibitors. Results demonstrated that phosphatidic acid, a highly selective inhibitor of PP1 [50], effectively blocked cell destruction by DHA, whereas lysophosphatidic acid, a structurally similar but inactive compound for PP1, did not prevent DHA-induced cell destruction (Table 1). Similarly, permethrin, an inactive structural analogue of cypermethrin (PP2B inhibitor), had no effect on DHA-induced cell destruction. Furthermore, phosphatidic acid also prevented pro-caspase-3 cleavage and PARP degradation (unpublished results). Moreover, DHA-induced activation of phosphatases is also evident from results shown in Fig. 7. These data clearly demonstrated that DHA caused activa-
tion of cellular phosphatases, which results in de-
phosphorylation of several cellular proteins, as evi-
dent on the autoradiograph (Fig. 7B). These data also demonstrate that the lower intensities of phos-
phorylated protein in the presence of DHA are not
due to different amounts of proteins in different lanes (Fig. 7A). In sum, these results suggest DHA causes activation of cellular phosphatases and that PP1- and

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<tr>
<td><strong>Effects of PP1 inhibitor on DHA-induced cell survival in Jurkat leukemic cells</strong></td>
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<tr>
<th>Cell viability (%) survival</th>
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<th>(+) DHA</th>
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<tr>
<td><strong>Control</strong></td>
<td></td>
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</tr>
<tr>
<td>Without serum</td>
<td>95 ± 5</td>
<td>37 ± 10***</td>
</tr>
<tr>
<td>With serum (2%)</td>
<td>95 ± 4</td>
<td>64 ± 6***</td>
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<tr>
<td><strong>Active inhibitors analogues</strong></td>
<td></td>
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<tr>
<td>25 µM phosphatidic acid* (PP1)</td>
<td>95 ± 2</td>
<td>76 ± 8NS</td>
</tr>
<tr>
<td>500 pM cypermethrin (PP2B)</td>
<td>96 ± 4</td>
<td>84 ± 5NS</td>
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<td><strong>Inactive inhibitors analogues</strong></td>
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<tr>
<td>25 µM lysophosphatidic acid* (PP1)</td>
<td>82 ± 5</td>
<td>6 ± 1***</td>
</tr>
<tr>
<td>500 pM permethrin (PP2B)</td>
<td>88 ± 5</td>
<td>38 ± 5***</td>
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*Cells (1 × 10^5/ml) were grown in the absence of serum with 15 µM DHA and active or inactive inhibitors of PP1 for 2 h or in the presence of 2% bovine calf serum with 60 µM DHA for 48 h with active or inactive inhibitors of PP2B. Cell viability was measured by the trypan blue exclusion assay. The results are mean ± S.E.M. for 3 experiments. Results are analyzed by Student’s t-test, relative to the (-) DHA group: ***P < 0.001; NS, not significant.
PP2B-type protein phosphatases play a role in DHA-induced cell destruction.

3.6. Activation of caspase-3 and degradation of PARP are prevented by PP1 and PP2B inhibitors

To study whether prevention of DHA-induced cell destruction by PP1 and PP2B inhibitors is due to their effects on the process of apoptosis, we assayed caspase-3 and PARP degradation in Jurkat cells. Consistent with the data on cell viability shown in Fig. 8, OKA weakly prevented DHA-induced procaspase-3 cleavage (36% control vs. 44% treated), whereas TMY (36% control vs. 68% treated) and CMT (36% control vs. 70% treated) effectively prevented pro-caspase-3 cleavage, and hence, activation of caspase-3 (Fig. 8a). Similarly, TMY (23% control vs. 80% treated) and CMT (23% control vs. 98% treated) also inhibited the degradation of PARP (Fig. 8b), a substrate of caspase-3, whereas OKA

![Fig. 8. Effect of protein serine/threonine phosphatase inhibitors on caspase-3 and PARP degradation. Cells (1×10^6/ml) were grown in the presence (gray bars) or absence (black bars) of 60 μM DHA and treated with either vehicle (control), 10 nM okadaic acid, 10 nM tautomycin, or 500 pM cypermethrin as described in the legend to Fig. 6. After each incubation, cells were lysed and then subjected to protein separation on a 12.5% SDS-PAGE. The separated proteins were blotted onto a PVDF membrane and immunoblotted. The caspase-3 or PARP bands were detected by Western immunoblotting using (a) anti-caspase-3 or (b) anti-PARP antibodies and ECL-chemiluminescence method. Amounts of procaspase-3 and non-degraded PARP were quantified by densitometric analysis using an HP ScanJet 4C scanner and Intelligent Quantiﬁer software (Bioimage Systems, Ann Arbor, MI). The results of DHA-treated values (grey bars) are relative to non-DHA-treated values (black bars).](https://example.com/fig8.png)
weakly prevented PARP degradation (23% vs. 38%) in Jurkat cells incubated with DHA. These results strongly implicate PP1- and PP2B-like protein phosphatases in DHA-induced apoptosis.

4. Discussion

In this study we demonstrate that DHA induces apoptosis in a human leukemic cell line and suggest that this fatty acid mediates its effects through activation of protein serine/threonine phosphatases. Cell viability using trypan blue exclusion assay, a widely used procedure in apoptosis studies [51,52], was initially used to examine the cytotoxic effects of DHA on Jurkat cells. However, other assays (caspase-3 activation, PARP degradation, DNA fragmentation) were used to demonstrate apoptosis as an end result of exposure to cytotoxic DHA in vitro. Our failure to see the cytotoxic effects of OA and AA on Jurkat cells compared to DHA under identical conditions may be due to either a direct effect of DHA on Jurkat leukemic cells or due to the differences in the uptake of these fatty acids or the rate of their degradation. These differences were not investigated in the present study as they are beyond the scope of this study. However, our results demonstrate that cell viability and cell apoptosis were unaffected by low concentrations of DHA and short incubation times, suggesting that pharmacological doses of DHA, achieved at the site of tumor growth, may be therapeutic, whereas lower doses are non-toxic. In the present investigation, ethanol was used instead of albumin to deliver DHA to cells. The concentration of ethanol did not exceed 0.05%, which was found to be non-cytotoxic. The amount of serum albumin in tissue cultures during incubation with DHA was estimated to be 10 μM, which gives an albumin:DHA molar ratio in the range of 1:3 (30 μM DHA) to 1:9 (90 μM DHA). When serum was completely removed, a concentration of 15 μM DHA was found to be cytotoxic to Jurkat cells (Table 1), which is 4 times less than the effective concentration (60 μM) in the presence of serum. These results indicate that serum may act as a reservoir where DHA is present bound to albumin, and in the absence of serum (albumin), a concentration of DHA 4 times lower is cytotoxic.

Very little is known about the molecular mechanism through which DHA may be cytotoxic for cancer cells. Our results suggest the involvement of a PP1/PP2B-type protein, serine phosphatase, in DHA-induced apoptosis of Jurkat cells. However, it is not clear from the present results if there are 2 different protein phosphatases involved or a unique protein phosphatase that is responsive to both PP1 and PP2B inhibitors. These studies also indicate that this PP1/PP2B-type protein phosphatase acts upstream of caspase-3 activation in the sequence of apoptotic events. Furthermore, it is also possible that there are multiple signaling pathways activated through a parallel PP1 and PP2B activation, converging at a common point. It is not obvious how DHA influences these protein phosphatases. This influence could be through a direct interaction of DHA with the regulatory units of these phosphatases or through generation of 1 or more secondary mediators. An important role for sphingoid lipids in the modulation of cell response to different extracellular signals has recently been uncovered, and a number of studies suggest an important role for ceramide in apoptosis [53,54]. It has been shown that ceramide activates a specific ceramide-activated protein phosphatase (CAPP), which is a member of the protein phosphatase 2A class of serine/threonine protein phosphatases [55–57], and that activation of this enzyme mediates the induction of apoptosis [57,58]. Furthermore, recent studies report that ceramide can also induce apoptosis through activation of class 1 protein phosphatase [45]. These findings, therefore, suggest a broader role for ceramide in regulating apoptosis through the activation of various protein phosphatases. Ceramide formation occurs in caveolae-like domains in plasma membranes that are rich in sphingomyelin [58]. Our previous work has indicated that DHA is rapidly incorporated into membrane phospholipids (particularly phosphatidylethanolamines) [59] and then alters membrane structure and function [60,61]. Our working hypothesis is that DHA incorporation into Jurkat cells, as investigated here, may alter membrane microdomains, resulting in the accumulation of sphingomyelinase into lipid rafts that are already enriched in cholesterol, saturated fatty acyl chains, and sphingolipids, including sphingomyelin. Enzymatic degradation of sphingomyelin to phosphorylcholine and ceramide would en-
sue, initiating the apoptosis cascade. This may be cell-type-specific, however, as DHA did not appear to affect ceramide-induced apoptosis in HL-60 cells [17].

One other possible mechanism of DHA-induced apoptosis in Jurkat cells is through a Fas-mediated process. Jurkat cells are known to express Fas and secrete Fas ligands [47,62–66]. It is possible that incubating cells with DHA results in increased expression of Fas and/or elevated secretion of Fas ligand, which in turn causes Fas-receptor-mediated apoptosis in these cells. However, this possibility is less likely because our initial experiments did not show activation of caspase-8, indicating the absence of a receptor-mediated process (data not shown). Another possible mechanism of DHA-induced caspase-3 activation leading to cell apoptosis through PP1A/PP2B is via interaction with mitochondria. Caspase-9 is regulated upstream by the release of cytochrome c from the mitochondria [44]. The interaction of cytochrome c with another apoptotic protein, Apaf-1, and dATP would also lead to caspase-3 activation. Further studies are clearly needed to explore these possibilities, among others, to determine the true path of apoptosis induced by DHA.

In conclusion, this research indicates that DHA in Jurkat leukemic cells induces many of the hallmarks of apoptosis on cytosolic and nuclear levels. Upon significant DHA exposure, cells activate the apoptotic caspase-3 enzyme, degrade the DNA repair enzyme PARP, and undergo DNA fragmentation as assayed by TUNEL flow cytometry analysis. These processes appear to be regulated upstream by activation of the PP1A/PP2B class of protein phosphatases.

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