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ORIGINAL PAPER

Dietary polyunsaturated fatty acids as inducers of apoptosis: implications for cancer

Simona Serini · Elisabetta Piccioni · Nicolò Merendino · Gabriella Calviello

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Abstract It has recently become clear the role played by alterations in apoptosis during the development of several chronic diseases (i.e. inflammatory, neurodegenerative and neoplastic pathologies). For this reason, the research for possible therapeutic strategies involving the modulation of the apoptotic pathways has attracted considerable interest in the past few years. In particular, it has been shown that apoptosis may be induced or inhibited by a variety of nutritional compounds providing health benefits. The aim of this review is to examine the ability of different dietary polyunsaturated fatty acids (PUFAs) to induce apoptosis, especially in the cancer field. The molecular effects of different PUFAs found in dairy products, meat, fish, vegetable seeds and oils, and known to affect the incidence and progression of cancer and other chronic diseases, will be analyzed. To this aim, our effort will concentrate in critically reviewing the published works concerning the effects of: (a) the n-6 PUFAs γ-linolenic acid, arachidonic acid, and conjugated linoleic acid; (b) the n-3 PUFAs eicosapentaenoic acid and docosahexaenoic acid on the apoptotic process. We will also pay attention to the recent findings regarding the possible role of PUFAs as regulators of the endoplasmic reticulum stress-pathway of apoptosis.

Keywords Apoptosis · γ-LNA · CLA · DHA · EPA · ER-stress

S. Serini · E. Piccioni · G. Calviello (✉)
Institute of General Pathology, Catholic University,
L. go F. Vito, 1, 00168 Rome, Italy
e-mail: g.calviello@rm.unicatt.it

N. Merendino
Department of Environmental Sciences, Tuscia University,
Viterbo, Italy

Introduction

It has recently become clear that different naturally occurring dietary polyunsatured fatty acids (PUFAs) and some of their synthesized derivatives are able to modulate the molecular pathways involved in apoptosis [1]. The critical analysis of PUFA action on these pathways appears particularly interesting, since alterations in apoptosis may be crucial for the development of several chronic diseases including inflammatory, neurodegenerative and neoplastic disorders [2–4]. In addition, it is largely known that the incidence and progression of these diseases may be strongly influenced by some PUFAs [5, 6]. Actually, as a whole, n-6 PUFAs have been widely recognized as factors worsening the development of these diseases [7]. However, a potential beneficial action on health has been reported for some of them [γ-linolenic acid (GLA), and conjugated linoleic acid (CLA)] [8, 9]. Also the n-6 PUFA arachidonic acid (AA), even though easily metabolized to a series of pro-inflammatory and pro-neoplastic eicosanoids, may exert pro-apoptotic and anti-neoplastic action when its oxidative metabolism is inhibited and it accumulates intracellularly in an unesterified form [10]. Finally, plenty of studies are available on the health benefits of the major n-3 PUFAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] [11]. In our laboratory we have been studying the antitumoral action of n-3 PUFAs for over 15 years, and, in particular, we have examined the pro-apoptotic action of DHA [12–16]. We recently found that DHA may act by modifying the expression of key molecules playing a crucial role in the induction of angiogenesis or apoptosis, such as COX-2, β-catenin, survivin, and the dual-phosphatase MKP-1 [12, 13, 15]. The major aim of the present review is to critically examine the pro-apoptotic effects of GLA, CLA, unesterified AA, EPA and DHA reported in different

kinds of cancer cells and tissues. In particular, the molecular pathways involved in their pro-apoptotic actions will be analyzed. Moreover, it will be also considered the protective effect exerted by these PUFAs against the development of other chronic diseases through their ability to regulate apoptosis in normal cells.

γ-Linolenic acid (GLA) and apoptosis

GLA (all-cis-6,9,12-octadecatrienoic acid, C18:3, n-6) is produced in the body from the essential PUFA C18:2, n-6 (linoleic acid, LA) by the enzyme Δ-6-desaturase. Only small amounts can be obtained directly from green leafy vegetables and nuts which contain traces of it. GLA is further elongated to dihomo-γ-linolenic acid (DGLA) which competes with AA for cyclooxygenase (COX)-1 and COX-2 pathways, inhibiting, on one hand, the production of the AA-derived prostaglandin (PG) PGE_2, known to exert pro-inflammatory and carcinogenic effects, and, conversely, producing PGE_1 known to exert anti-inflammatory activities in a variety of systems and disease conditions. Moreover, via the 15-lipoxygenase (15-LOX) pathway, DGLA is oxygenated to 15(*S*)-hydroxyeicosatrienoic acid (15-HETrE) [17], an inhibitor of 5-LOX-induced formation of pro-inflammatory compounds LTB_4, LTC_4 and LTD_4 from AA [17].

Many papers have shown that GLA and its metabolites may affect the expression of various genes which codify products playing crucial roles in the regulation of apoptosis [8, 18]. On this basis we decided to critically analyze the studies concerning the effects of this fatty acid on cell growth and survival dividing them between the in vivo and in vitro studies.

In vitro studies: tumor cells

A series of in vitro studies have showed that GLA and its derivatives inhibit selectively the growth of tumor cells [19–21]. Induction of apoptosis is considered to be mainly responsible for this effect. Different molecular alterations produced by GLA in the cells have been related to its pro-apoptotic effect observed in vitro:

(a) The GLA ability to enhance the generation of lipid peroxidation products and free radicals has been considered to be the most likely molecular mechanism for its cytotoxic and pro-apoptotic effects in different cancer cells [21–27]. Interestingly, it has been hypothesized [25] that it is not the GLA-induced cellular oxidative stress per se that can explain the pro-apoptotic effect of GLA, but the specific ability of GLA and/or its peroxidized products to bind DNA. According to this hypothesis, also the higher cytotoxic efficiency of GLA compared to other more unsaturated fatty acids can be easily explained. The binding of GLA itself or its peroxidized derivatives to DNA has been invoked to explain the suppression of the anti-apoptotic ErB-2 oncogene expression [26], and its pro-apoptotic effect in several kinds of cancer cell lines (breast, ovary and gastrointestinal cancer cell lines) [27].

(b) Another interesting hypothesis put forward to explain the pro-apoptotic effect of GLA in Hep2 human larynx tumor cells [28] was related to the inhibition of the mitochondrial carnitine palmitoyltransferase I (CPT I) activity and subsequent fatty acid oxidation exerted by this fatty acid in these cells.

(c) Moreover, it has been suggested that GLA and GLA derivative-induced alterations in eicosanoid metabolism may play a key role in the cytotoxic action of GLA [29]. However, this study, conducted in human cervical carcinoma HeLa cells, did not verify if the cytotoxic effect exerted by GLA could be specifically ascribed to an increase in apoptosis.

Concluding this section, it is important to underline that the papers analyzed generally agree that the cytotoxic and pro-apoptotic effect of GLA is highly selective for cancer cells, even though also normal cells or differentiated cancer cells are sensitive to its pro-apoptotic action [30]. However, normal cells were shown to be less sensitive to the cytotoxic action of GLA [31] and, for instance, whereas as low as 18 μM GLA was enough to efficiently induce apoptosis in lymphocytic leukemia cells, at least 108 μM GLA was needed by lymphocytes from normal individuals. In agreement, it has been reported that GLA is able to induce apoptosis in normal and transformed fibroblast and lymphoblast cell lines, but also in this case normal cells were less responsive than cancer cells [30]. However, there is not general agreement on this point, and it has been reported that GLA efficiently and similarly induces apoptosis both in HL-60 promyelocytic leukemia cells [32], but also in DMSO-differentiated HL-60 cells [33] and in C3HA normal murine fibroblasts [34]. Likewise, GLA induces apoptosis in normal human umbilical vein endothelial cells (HUVEC), arresting them in G0/G1 phase [35], even though the GLA concentration used (300 μM) in this work is more elevated than those generally used (about 30–150 μM) in the other in vitro studies.

Analyzing all the papers reporting the pro-apoptotic effect of GLA, it was possible to establish that 10–60 μM was the range of concentrations at which GLA was generally effective in inducing apoptosis in the different kinds of cancer cells. These values may be easily achieved in vivo, as demonstrated by Surette et al. [36], who observed a basal GLA concentration of 35 μM in human plasma, which increased by 43% after a supplementation with 1.5 g GLA/die for 3 weeks. These observations suggest that the GLA pro-apoptotic effects observed in vitro may have a

relevance also in vivo. That, nevertheless, is indicated also by the results of the animal studies reported below, which directly demonstrated the efficacy of in vivo treatments with GLA in inducing apoptosis and inhibiting growth of tumor cells.

In vivo studies: animal models of cancer

Most of the in vivo studies with GLA have been performed on gliomas. It was shown [37] that GLA is able to inhibit growth, induce apoptosis and decrease proliferation in C6 glioma cells implanted in rats. Moreover, it has been reported that GLA and γ-irradiation synergistically induce apoptosis and cause regression of gliomas, preserving, however, normal neural tissue and vasculature in adjacent brain [38]. This finding is of great interest, suggesting the possible use of GLA for the inhibition of residual cell proliferation and migration after surgical removal of the glioma mass [39]. Similarly, inhibition of growth and induction of apoptosis has been observed in Walker 256 carcino-sarcoma transplanted in rats treated with a diet at high levels of GLA (5.5% for 12 days) [40]. In this in vivo study the pro-apoptotic and anti-tumoral effect of GLA was partly ascribed to increased levels of lipid peroxides in tumor cells.

On the whole, in light of the often contrasting findings obtained in vitro, we may conclude that further work is certainly required to definitely ascertain the selective toxicity of GLA towards neoplastic cells by using a more ample spectrum of cancer cell lines in vitro. Also, more effort is needed to establish the in vivo doses allowing a safe use of this fatty acid against cancer, without affecting normal tissues.

Conjugated-LA (CLA) and apoptosis

Plenty of studies have investigated the beneficial effect of CLA against atherosclerosis, hypertension, diabetes, inflammation, and some kinds of cancer [for a review, see 9]. CLA differs from the parent LA, whose adverse health effects are widely documented, since it does not possess methylene groups between the double bonds. In CLA conjugated double bonds in either cis or trans configurations are present in various positions to give different geometric and positional isomers. The most abundant isomer found in nature, especially in meat and dairy products, is the cis-9, trans-11 (c9,t11) CLA isomer, originating in ruminant tissues from Δ-9 desaturation of vaccenic acid (18:1 trans-11) [41] and, to a lesser extent, from bacterial hydrogenation of LA in the rumen [42]. However, the CLA commercially available and mainly used in investigations is a mixture of equal proportions of the major c9,t11-CLA and trans-10, cis-12 (t10,c12)-CLA isomers, accompanied by other isomers in lower amounts. Distinct effects on apoptosis have been reported for the CLA mixture and for each of the two major isomers. For this reason, and for the sake of clarity, we will indicate the mixture of isomers as "CLA" and will use the names c9,t11; t10,c12 or trans 9, trans 11 (t9,t11), to specifically indicate the different kinds of isomers used.

In vitro studies: tumor cells

The pro-apoptotic effect of CLA and its individual isomers has been reported for a variety of cancer cell lines in vitro. Multiple studies have tried to identify the molecular pathways through which CLA-induced apoptosis takes place. We will firstly (sections "Molecular pathways of CLA-induced apoptosis", "Endoplasmic Reticulum (ER) stress-induced apoptosis: a novel pathway of apoptosis modulated by CLA in cancer cells") analyze the molecular pathways of apoptosis which may be induced by CLA. Thereafter, we will examine the apoptosis-inducing ability of CLA and of its diverse isomers in different kinds of cancer cells. Since the majority of the studies were performed on breast and colon cancer cell lines, we will firstly analyze these studies (sections "Breast cancer cells", "Colon cancer cells"), and then those carried out on a variety of other strains of cancer cells (section "Other cancer cells"). In the last three sections particular attention will be paid to the analysis of the signaling transduction pathways induced by CLA and its isomers upstream the apoptotic response.

Molecular pathways of CLA-induced apoptosis

Studies with a variety of cancer cells demonstrate that CLA activates mainly an intrinsic pathway of apoptosis [43, 44]. In different breast cancer cell lines this was proven by alterations induced by CLA in the expression of different proteins of Bcl-2 family, including Bax, Bcl-2, Bcl-xL,and Bak, all in a pro-apoptotic sense, as well as by the translocation of cytochrome *c* from mitochondria and the cleavage of caspase-9. Interestingly, however, the mitochondrial pathway was differently regulated in breast cancer cells differing for the p53 status. In p53 wild-type cells the induction of apoptosis was related to the increased expression of p53 and the decreased expression of Bcl-2 [43], while in the p53-mutant MDA-MB-231 CLA increased the levels of the pro-apoptotic protein Bcl-xS and Bax [43]. These findings indicated that p53 activation may be involved in CLA-induced apoptosis, but is not essential. In agreement, CLA and both the two individual c9,t11, and t10,c12 isomers caused an increase in caspase-9 activity, the release of cytochrome *c* from mitochondria and the

reduction of Bcl-2 expression in colon SW480 tumor cells [45]. Moreover, it was recently observed [46] that in Caco-2 colon cancer cells the *all trans* t9,t11 isomer acts as a powerful inducer of apoptosis decreasing the levels of Bcl-2. Likewise, caspase-9 was induced by CLA in ADF glioblastoma [47] and by t10,c12 in rat hepatoma 4dRLh-8a cells [48], in which the increase of cleaved Bid and translocation of Bax protein into the mitochondrial membrane were also observed. A decrease of Bcl-2 was also induced by t10,c12 in PC-12 prostate cancer cells [49] and by c9,t11 in SGC-7901 gastric cancer cells [50]. All these findings univocally indicate that the intrinsic apoptotic pathway was preferentially induced by this fatty acid, whatever the cancer cell model used. Also, a mixture of trans, trans isomers was recently found to be even more effective than the major c9,t11 and t10,c12 isomers in the induction of a mitochondrial-mediated pathway of apoptosis in human MG-63 osteosarcoma cells [51]. Conversely, however, it was also found that the c9,t11 isomer caused an increase of FAS/CD95 protein in the SGC-7901 gastric adenocarcinoma cells together with a decrease in Bcl-2 levels [50], suggesting the parallel activation of the extrinsic and intrinsic pathways of apoptosis. Actually, also in SK-HEP-1 hepatoma cancer cells CLA stimulates both the extrinsic and intrinsic pathways of apoptosis, as shown by the activation of both caspase-8 and -9 [52]. It is possible that the discrepancies observed in the last two studies may be related to the particular cell models (gastric carcinoma and hepatoma cells) used. Figure 1 shows schematically the molecular pathways of apoptosis whose components have been shown to be regulated by CLA or/and its individual isomers in cancer cells, including the ER stress-apoptosis pathway described below.

Endoplasmic reticulum (ER) stress-induced apoptosis: a novel pathway of apoptosis modulated by CLA in cancer cells

The recent discovery that t10,c12 may exert a pro-apoptotic effect in TM4t rat breast cancer cells through the activation of a ER stress response appears worth noting [53]. In our opinion, this emerging research area will attract considerable attention in the next future. The pathways involved in the ER stress-induced response are currently under study, and their analysis is beyond the aim of the present review. But, given the relative novelty of the subject, a brief description of the most important ER stress-induced pathways seems crucial to favor a better comprehension of the CLA effects on them. Various cellular stresses which perturb the highly specialized protein synthesis and folding activities of ER may lead to accumulation of misfolded or unfolded proteins in ER lumen [54]. As a consequence, a survival pathway is activated, i.e. the unfolded protein response (UPR), with the aim to restore the perturbed ER homeostasis and re-establish normal ER functions [55, 56]. However, also apoptosis may be induced as a result of long lasting or extremely intense ER stress [57]. In non-stressed cells the ER chaperone GRP78 binds to the ER transmembrane sensor proteins PERK, IRE1 and ATF6 and maintains them in an inactive form [54]. The UPR pathways initiated by these sensor proteins become active when excessive amounts of unfolded proteins in ER lumen bind to

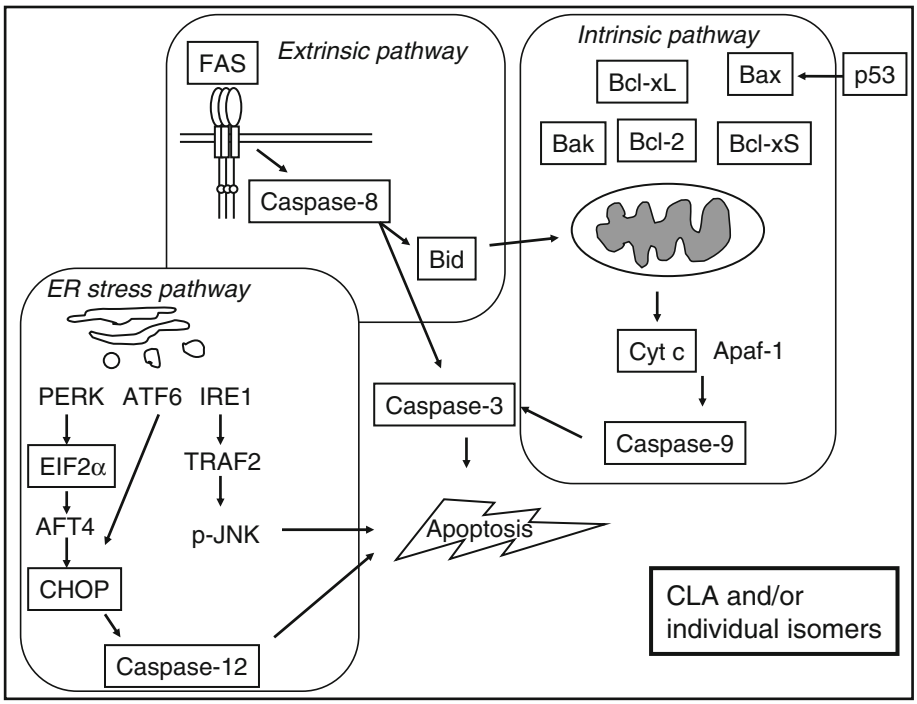

Fig. 1 Molecular pathways of CLA-induced apoptosis in cancer cells. The molecules involved in the different pathways of apoptosis and whose expression or activities are regulated by CLA (i.e. a mixture of different CLA isomers) or/and the individual isomers are enclosed in *boxes* (see the text for more details). In the scheme the apoptotic pathways are simplified and include only those components necessary to explain the pro-apoptotic action of CLA and its isomers

GRP78 and move it away. Consequently, various molecular factors are modified, becoming inactivated (i.e. the initiation factor 2, eIF2) or expressed at higher levels (the transcription factors ATF4 [58]; the pro-apoptotic protein CHOP [59]; the X-box-binding protein-1, XBP-1 [60]). In particular, the increase in CHOP transcription, occurring as the ER stress becomes too protracted or intensive, is thought to represent the switch from the UPR survival pathways to the ER-induced apoptotic response [54]. Moreover, active IRE1 may recruit the apoptosis-signal-regulating-kinase 1 (ASK1) which activates JNK downstream and, consequently, the apoptotic response [54, 61]. Especially during prolonged ER stress, PERK, ATF6 and IRE1 can trigger pro-apoptotic signals, activating molecules such as CHOP or JNK which further drive cells down the death pathway. For instance, CHOP may down-regulate Bcl-2 and promote the activation and translocation of Bax [62]. JNK targets Bcl-2 and BH3-only members of the Bcl-2 family playing a role in ER stress, resulting in a pro-apoptotic effect. Among the caspases linked to ER stress-induced apoptosis, rodents caspase-12 seems to be a crucial mediator of the execution phase in ER stress-induced apoptosis. Caspase-4, present in human cells, is thought to have similar functions to caspase-12 [63]. The innovative hypothesis that an ER stress-driven apoptosis may be involved in the action of the t10,c12 CLA isomer has been proven by the observation that it induces the protein CHOP, and that knocking-down CHOP gene attenuates t10,c12-induced apoptosis. Moreover, t10,c12 induces the cleavage of the ER resident caspase-12 and a selective inhibitor of caspase-12 significantly alleviates t10,c12-induced apoptosis. t10,c12 induces also the overexpression and activation of XBP1, as well as the phosphorylation of EIF2α. These findings are of great interest, since for the first time new light is thrown on the possibility that PUFAs may induce apoptosis following an unusual ER stress-dependent pathway. Even more intriguing, this pathway was activated by t10,c12 in cancer cells, which often become resistant to the usual apoptosis pathways and may easily undergo ER stress for their elevated glycolitic metabolism and easy exposition to hypoxia [64, 65].

Breast cancer cells

It has been demonstrated that CLA may activate apoptosis in breast cancer cells, and a possible mechanistic role for the reduced phosphorylation of ERK has been suggested [43, 44]. Moreover, further studies have allowed to discriminate the specific effects exerted by the two major CLA isomers in these cells. It has been reported that both t10,c12 and c9,t11 isomers [66] may exert anti-proliferative and pro-apoptotic effects in different breast cancer cell lines in vitro. In several studies, however, it has been demonstrated the higher efficacy of t10,c12 isomer [46, 49, 67, 68]. Interestingly, this isomer appears particularly effective in inducing apoptosis with a caspase-9-dependent pathway in p53-mutant cells [69]. It has been recently observed that the estrogen receptor-α (ERα) plays a crucial role in the induction of the t10,c12-driven apoptosis in human breast cancer cells [70]. The t10,c12 isomer induced apoptosis in ERα-transfected MDA-MB-231 cells, but not in the wild type cells lacking the receptor. This finding suggests that the ERα-negative human breast tumors may be insensitive to t10,c12 treatment in vivo. This possibility is worth noting, since about 25% of human breast cancers are ERα-negative, but for the remaining 75% ERα-positive breast tumors the data would indicate a possible chemopreventive and therapeutic action of this isomer.

Colon cancer cells

Studies conducted in colon cancer cells have recently allowed to better understand the diverse ability of different isomers in inducing apoptosis. In particular, the higher efficiency of t10,c12 as compared to the c9,t11 isomer in inhibiting cell proliferation and inducing apoptosis was observed also in different colon cancer cell lines [45, 67, 68, 71]. However, it was recently observed [46] that the *all trans* t9,t11 isomer had an even higher efficiency than both the major c9,t11 and t10,c12 isomers in inducing apoptosis in Caco-2 colon cancer cells. On the whole, all the results obtained using colon cancer cell lines have added interesting information regarding the possible transduction pathways induced by the CLA isomers upstream the activation of cell proliferation and apoptosis. The two major isomers were found able to trigger molecular pathways involving IGFII, an autocrine growth stimulator of colon cancer cells [67], ERB3 signaling ad PI3-kinase/Akt pathway [68], or ATF3/Non Steroidal anti inflammatory drug Activated Genus-1 (NAG-1) and Akt/GSK-3beta [71]. One possible hypothesis to explain such ample variety of molecular pathways regulated by CLA and its individual isomers in cancer cells could be the possibility that CLA and its separate isomers may initially act through more general, unifying, mechanisms of action, leading to perturbations of the cellular microenvironment and, thereafter, as a consequence, to a variety of resulting molecular responses. For instance, CLA-induced modification of lipid peroxidation, tissue fatty acid composition, and eicosanoid formation [72] could be regarded as the possible early alterations induced by CLA and its individual isomers in cancer cells.

Other cancer cells

Further information about the mechanisms through which CLA may activate apoptosis has been obtained using other kinds of cancer cells. It was shown that CLA inhibited the

growth of a series of human cancer cells lines (hepatoma, prostate, glioblastoma, bladder) [73] and, worth noting, whereas cell proliferation was always reduced by CLA, apoptosis response was activated only in the most deviated cells. Various studies carried out on different cancer cells have allowed to identify molecular factors involved in the transduction pathways activated by CLA upstream the apoptotic response. Among these factors, some nuclear receptors belonging to the family of the peroxisome proliferator activated receptors (PPAR) are worth noting, being involved in the transduction pathways related to cell survival and death. In particular, PPARα, PPARβ/δ and PPARγ were found modulated by CLA in different cancer cell lines, including hepatoma cells, prostate cancer, glioblastoma, bladder cancer and breast cancer cells [47, 73]. The CLA-dependent activation of type 2A phosphatase (PPA2) in SK-HEP-1 hepatoma cells [52] seems also a relevant pro-apoptotic transduction signal since it was directly related to the dephosphorylation of Bcl-2 and to the increased activity of Bax and Bad.

Finally, to conclude this section, it is interesting to observe that the data published in most of the papers demonstrate that the concentrations of CLA or its isomers effective in inducing the apoptosis of different strains of cancer cells in vitro were comprised approximately in the 1–50 μM range. It has been reported by different authors [74, 75] that the basal plasma level of CLA in humans is about 20 μM, and after a CLA supplementation (350–500 mg/die, containing 50% c9,t11, and 50% t10,c12, for 8 weeks) its concentration increases in plasma by about 75% (about 35 μM). Thus, levels of CLA similar to the optimal concentrations generally used to obtain apoptosis are easily achievable in plasma, suggesting that the pro-apoptotic effects reported in the in vitro studies may be actually relevant also in vivo. However, the direct demonstration of CLA capability to inhibit tumor growth in vivo by altering apoptosis has been obtained through a series of studies on animals subject to chemical carcinogenesis, transplanted with tumors or representing genetic models of cancer (see below).

In vivo studies: animal models of cancer

It has been observed that a dietary supplementation with CLA, purified t10,c12 or c9,t11 (all at 1%) in rats subject to 1,2-dimethylhydrazine (DMH)-induced carcinogenesis reduced the incidence and growth of colon cancer by induction of apoptosis in colonic mucosa cells [76, 77]. A mechanism considered responsible for the pro-apoptotic effect exerted by CLA was the change in levels of AA and its oxygenated metabolites (eicosanoids) in the colonic mucosa cells [76]. Once more, the increased Bax/Bcl-2 ratio found in tumor tissues confirmed the preferential CLA-induction of the mitochondrial apoptotic pathways. However, other results obtained on animals bearing colon tumors lacked to find an anti-carcinogenic and pro-apoptotic role for CLA [77], and in one case t10,c12 showed even a pro-carcinogenic effect [78]. It is possible that these discrepancies may be related to the use of different CLA mixtures of isomers, since each isomer used individually may have a specific effect on survival and death of tumor cells, whereas may lead to different effects if used in combination with other isomers. Also, the exact composition of the mixture should be considered, because its effect may vary in relation to the relative percentage of each of the two major isomers (c9,t11 and t10,c12), or of other isomers generally defined "minor", which, however, may exhibit strong effects on tumor growth. Moreover, the effect may be related also to the particular kind of tumor and to the genetic features of the tumor cells. For instance, it has been shown that the individual administration of either c9,t11 or t10,c12 with the diet (0.5% for 6 or 24 weeks) inhibited at the same degree the initiation and progression stages of methylnitrosourea (MNU)-induced rat mammary carcinogenesis [79] and the metastatic tumor burden of mammary tumor cells transplanted in syngeneic mice [80]. However, recently, it was found that t10,c12 was able to stimulate mammary tumorigenesis in transgenic mice overexpressing erbB2 [81]. Therefore, the higher level of this oncogene protein in mammary cells may change the cellular response to t10,c12. This observation is highly relevant since over-expression of erbB2 is observed in 20–30% of human breast cancers, and is associated with cancer progression and a poor prognosis [82]. Moreover, since in this study t10,c12 increased the proliferation of mammary epithelium not only in transgenic mice, but also in wild mice, the possible risk involved in its supplementation should be carefully considered [81]. On the other hand, apoptosis was increased in this model of breast cancer, but the proliferative effect was prevalent [83] confirming in vitro data on breast cancer cells [69]. The enhanced tumor growth was associated with increased phosphorylation of the IGF-I/insulin receptor, as well as with increased signaling through the mitogen-activated protein kinase (MEK)/ERK and phosphatidylinositol 3-kinase/Akt pathways, providing important information concerning the proliferative signaling triggered by t10,c12 upstream cell proliferation and apoptosis in these cells [83].

Another isomer, the trans, trans isomer t9,t11 was recently observed to inhibit the development of azoxymethane (AOM)-induced colonic aberrant crypt foci in rats [84] by inducing apoptosis and suppressing cell proliferation in the non-lesional crypts. This finding confirms the powerful pro-apoptotic effect of a mixture of trans, trans isomers observed in human MG-63 osteosarcoma cells and reported above [51]. Moreover, it strongly suggests that

other "minor" isomers should be considered, since they could have even more powerful effects on tumor growth than those observed with each of the two major isomers (c9,t11 and t10,c12).

CLA and apoptosis in other fields of human health

The pro-apoptotic action of CLA may result in health benefits other than the inhibition of tumor growth [85–87]. These findings are remarkable since demonstrate that the pro-apoptotic effect of CLA may have a more general protective significance. The following beneficial effects, will be briefly analyzed: (a) CLA anti-obesity effect, (b) CLA-induced liver preservation from fibrosis, and finally (c) CLA capability to promote the regression of pre-established atherosclerosis.

(a) CLA has been reported to be able to reduce body fat and enhance lean body mass in several animal models and in humans [75, 88]. This effect has been attributed to the t10,c12 isomer [89] and ascribed to the apoptosis of adipocytes [85, 90, 91]. Among the molecular mechanisms involved in the pro-apoptotic action of CLA in adipocytes there are the overexpression of TNF-α and of the Uncoupling Protein-2 (UP2) [85]. Much more effort, however, should be expended to clarify whether CLA-induced apoptosis may be mechanistically related to CLA-induced body mass reduction, since not all the evidence sustains this hypothesis. For instance, it was proven the direct relationship between the metabolism of CLA through elongation and desaturation and the reduction of body mass, but the induction of apoptosis appeared unrelated to the desaturase and elongase metabolism of CLA [92].

(b) The protective effect of dietary CLA (1% of the diet) was also demonstrated in a rat fibrosis model induced by carbon tetrachloride [86]. CLA protected against liver damage and a reduced formation of collagen fibers in liver. It was observed that the c9,t11 isomer induced apoptosis of rat hepatic stellate-T6 (HSC-T6) cells in vitro [86]. The authors suggested that this effect observed in vitro could represent the protective mechanism through which CLA may preserve liver from fibrosis in vivo, since during the hepatic damage, HSCs transform into myofibroblasts producing extracellular matrix materials such as collagens, fibronectin and proteoglycans [93].

(c) CLA supplementation has been observed to cause regression of pre-established atherosclerosis in the mouse ApoE (−/−) model through the increased apoptosis of cells in the atherosclerotic lesions [87]. The effect observed in vivo with CLA was also related to the apoptosis induced by both the major CLA isomers in THP-1 macrophages in vitro [87]. The intense stimulation of intracellular ROS production observed in macrophages treated in vitro with the major CLA isomers [94] was considered by the authors as the main cause of apoptosis, in agreement with the hypothesis that CLA effects may be mediated by the enhanced cellular peroxidation and generation of ROS [94]. Interestingly, however, on the basis of the known anti-inflammatory properties of CLA, it had been previously suggested that this fatty acid could be used for the prevention of ischemic hearth disease [95]. Since, in addition, the pathogenesis of atherosclerosis has been largely related to an increased oxidative stress, rightly the authors suggest a critical reconsideration of the possibility to include CLA in the prophylaxis of ischemic disease.

Unesterified AA and apoptosis

It is known that AA is released from membrane phospholipids and metabolized to eicosanoids (i.e. PGs, LTs and lipoxins) as a response to a series of stimuli [96]. Most of these metabolites are biologically active lipids exerting potent pro-inflammatory actions [97] and are recognized as positive key regulators of cell survival, neoangiogenesis and metastasis [98, 99]. For this reason, agents that specifically target these lipid mediators or the enzymes that catalyze their formation starting from AA (COX and LOX) have been investigated as potential anticancer drugs [100]. On the other hand, it has been observed that the addition of exogenous unesterified AA to different cancer cell lines inhibits cell growth and induces apoptosis [101]. It has been suggested that this effect exerted by free AA may be strictly related to the overexpression of COX-2 in many tumors, since it is believed that COX-2 overexpression and that of other AA-metabolizing enzymes (i.e. LOX) may exert a crucial role in carcinogenesis [102, 103], providing an apoptotic escape for cancer cells through the decrease of intracellular AA levels. However, the COX- and LOX-induced decrease of AA can be overcome by the addition of exogenous AA to cancer cells in vitro [101]. For these reasons, it has been suggested [101] that the antitumoral and pro-apoptotic effect exerted by the inhibitors of COX-2 may be related to the accumulation of free, non-metabolized AA in cancer cells, and that the inhibition of free AA conversion to eicosanoids may represent a promising target for cancer therapy. However, the conversion of AA to eicosanoids should not be considered the only possible metabolic strategic target for anticancer therapy devoted to enhance the intracellular levels of free AA, but the reacylation/deacylation cycle of membrane phospholipids (Lands cycle) should be also taken into account. This mechanism controls the cytosolic free AA availability and its perturbation has been found to be involved in the induction of apoptosis in U937 human leukemia cells [104].

Molecular mechanisms involved in the pro-apoptotic effect of unesterified AA

On these bases, it seems crucial to examine the molecular pathways of free AA-induced apoptosis. Only a few works have been focused on this aspect and, therefore, more effort should be expended to clarify this point. Several reports demonstrated that AA induces a caspase-3 dependent apoptosis [101, 105]. The involvement of a mitochondrial pathway of apoptosis has been demonstrated by the release of cytocrome *c* in different kinds of rat hepatoma cells [106, 107] and neurons [105]. In MH1C1 hepatoma cells it was observed that [106] AA induced mitochondrial permeability transition (PT) and cytochrome *c* release affecting directly the PT pore, rather than through a decrease of the mitochondrial membrane potential. Also the sphingomielyn–ceramide pathway of apoptosis has been found to be activated by unesterified AA in TNFα-treated HL-60 cells [108]. Thus, due to the relatively few studies which have so far specifically addressed the molecular pathways of AA-induced apoptosis, it is not possible to understand whether a prevalent pathway of apoptosis exists or not.

Regarding the molecular modifications triggered by unesterified-AA upstream the apoptotic pathways, the induction of cellular oxidative stress, demonstrated by the increased formation of the lipid peroxidation products and by the reduced intracellular levels of glutathione, were observed in human retinoblastoma cells undergoing AA-induced apoptosis [109]. Moreover, recently, some possible molecular pathways affected by unesterified-AA and possibly leading to apoptosis, were identified by gene array analysis in HCT-116 colon cancer cells [101]. Exogenous AA was shown to down-regulate a number of genes known to play a role in cell survival and apoptotic resistance, including genes whose products act as growth factors (i.e. BDNF), growth factor receptors (i.e. fibroblast factor receptor), signal transduction molecules [i.e. SKP2 (P45)] and proteins involved in DNA and RNA metabolism (i.e. topoisomerase 2A). On the other hand, among the up-regulated target genes some coded for AP-1 protein and for EGR-1 zinc finger, a protein interacting with c-Jun in neuronal apoptosis, suggesting that the main pathway through which AA induces apoptosis may involve the activity of the AP-1 transcription factor. A number of the AA-upregulated genes coded also for proteins counteracting the ras/ERK/MAPK signaling, known to be implicated in carcinogenesis. Moreover, unesterified AA induces cytosolic and mitochondrial Na^+ and Ca^{2+} overload in neurons [105], which have been considered important upstream signals in the mitochondrial-mediated apoptotic pathway. Overall, we are still far from a clear picture of the molecular effects induced by AA upstream apoptosis both in normal and in cancer cells, and further work is required to better clarify this issue.

n-3 PUFAs and apoptosis

It has been widely recognized that the major n-3 polyunsaturated fatty acids EPA and DHA may exert beneficial effects in inhibiting the incidence and development of a series of chronic diseases, including cardiovascular, neurodegenerative, immune and inflammatory disorders, as well as cancer [110–113]. The regulation of the apoptotic pathways represents one of the biological mechanisms thought to be responsible for their beneficial effects. Whereas induction of apoptosis has been invoked to explain n-3 PUFA benefits on immune system and against hypertension and cancer [6, 114, 115], conversely, the inhibition of apoptosis in central nervous system and at endothelial level seems to play a crucial role in the protection exerted by n-3 PUFAs against chronic neurodegenerative diseases and atherosclerosis [116, 117].

In vitro studies

A number of studies carried out on cultured cells in vitro have demonstrated the pro-apoptotic effect of EPA or DHA. These studies were mainly performed on cancer cells, and have allowed to identify the molecular pathways through which n-3 PUFA-induced apoptosis may take place. We will firstly (section "Molecular pathways of n-3 PUFA-induced apoptosis") analyze the induction of these molecular pathways, and then we will examine the ability of n-3 PUFAs to modulate apoptosis, particularly in breast (section "Breast cancer cells") and colon cancer (section "Colon cancer cells") cell lines, which are the most used cell models. The effects observed in other kinds of cancer cells and in normal cells will be also examined (section "Other cancer cells"). The signaling transduction pathways which have been reported to be induced by n-3 PUFAs upstream the apoptotic response will be analyzed in the sections "Breast cancer cells", "Colon cancer cells", "Other cancer cells".

Molecular pathways of n-3 PUFA-induced apoptosis

The available data seem to indicate that apoptosis induced by EPA or DHA may follow both the extrinsic and intrinsic pathways. In some cells, such as lymphoma Ramos cell line, caspase-3 and -9 but not caspase-8 activities were increased by EPA [118]. These data, indicating a possible involvement of the intrinsic pathway, are in agreement with the findings obtained in a series of colon cancer cell lines (LS-174, colo 201, HT-29 and Caco-2) [14, 119, 120], demonstrating that

dietary DHA [14, 119] or fish oil [120] modify the expression of Bcl-2 family proteins, by increasing the levels of the pro-apoptotic proteins Bak and Bcl-xS and reducing those of the anti-apoptotic proteins Bcl-2, Bcl-xL [14, 119, 120]. However, in human Caco-2 colon cancer cells [121] DHA is able to increase the expression of both the caspases 8 and 9, and the activation of cytochrome *c*. Similarly, in HL-60 leukemia cells EPA induces cytochrome *c* release from mitochondria and mitochondrial membrane depolarization [122], but enhances also the activities of both caspases 8 and 9. In this case also the expression of cleaved Bid was enhanced, demonstrating a strict connection between the DHA-induced activation of caspase-8 and the activation of the mitochondrial pathway. The picture is even more complicated if we consider that DHA was also observed to sensitize [123] or inhibit [124] FAS-mediated apoptosis in HT-29 colon cancer cells and in U937 monocytic leukemia cells, respectively. Moreover, n-3 PUFAs have also been shown to trigger the sphingomielyn–ceramide pathway of apoptosis by causing a marked increase in neutral sphingomyelinase activity and ceramide production in MDA-MB-231 breast cancer cells [125] and in Jurkat leukemia cells [126]. Conversely, a decreased level of ceramide was shown in normal T lymphocytes treated with n-3 PUFAs [127, 128]. Overall, from these findings it appears clear that it still remains to be established which pathway of apoptosis may be induced by n-3 PUFAs. This fact may be related partly to the different actions that these fatty acids can exert in diverse cellular models, but also to the different n-3 PUFAs used (EPA, DHA or fish oil), as well as to the different ways of administration. Figure 2 shows schematically the molecular pathways of apoptosis whose components have been shown to be regulated by EPA or/and DHA in cancer cells.

Breast cancer cells

EPA and DHA have been recently observed to induce apoptosis in MDA-MB-231 human breast cancer cells, possibly by decreasing signal transduction through the Akt/NFkappaB cell survival pathway [129], or by altering lipid raft composition, decreasing epidermal growth factor receptor (EGFR) levels in lipid rafts, and increasing the phosphorylation of both EGFR and p38 [130]. Moreover, it has been shown [131] that DHA induced apoptosis in MCF-7 human breast cancer cells by the activation of PPARγ and consequent up-regulation of syndecan-1 (SDC-1), the major proteoglycan produced by epithelial cells, strategically placed in the plasma membrane to regulate growth factor signaling and cell–cell and cell–matrix interactions. Worth noting, often the mechanisms hypothesized for the apoptotic effect of n-3 PUFAs in breast cancer cells [130, 131] involve modifications of the plasma membrane environment, due to alterations of the molecular composition of lipid plasma membrane microenvironment (rafts), or to the increased expression of a plasma membrane molecule (SDC-1) playing a crucial role in the regulation of cell growth molecular signaling.

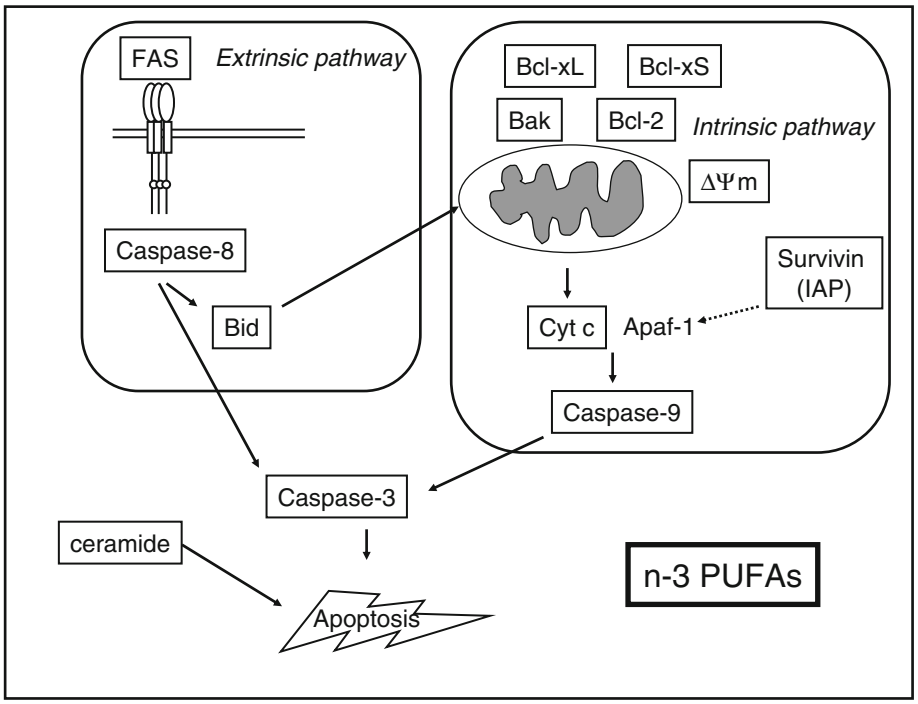

Fig. 2 Molecular pathways of n-3 PUFA-induced apoptosis in cancer cells. The molecules (or factors) involved in the different pathways of apoptosis and whose expression or activities are regulated by EPA and/or DHA are enclosed in *boxes* (see the text for more details). In the scheme the apoptotic pathways are simplified and include only those components necessary to explain the pro-apoptotic action of EPA and/or DHA

Colon cancer cells

Colon cancer cells have been shown to be generally sensitive to the pro-apoptotic effect of n-3 PUFAs [14, 121, 132, 133]. Moreover, it has been reported that down-regulation of COX-2 may be a crucial mechanism underlying the apoptotic effect of n-3 PUFAs in colon cancer cell [134]. Since COX-2 is known to confer resistance to apoptosis [135] and is overexpressed in colon cancer [136], the efficacy of n-3 PUFAs in inhibiting the expression of this enzyme appears crucial to explain the antitumor effect of these fatty acids in colon cancer [6]. Moreover, we recently reported that DHA induces apoptosis in human colorectal cancer cells not expressing COX-2 by the proteasome-dependent degradation of β-catenin and down-regulation of the anti-apoptotic factor survivin [13]. These results are really worth noting, since dysregulation of Wnt signaling and β-catenin expression is believed to be central in the early stage of sporadic carcinogenesis in humans and recent work suggests that the Wnt/β-catenin regulates the apoptotic process in colorectal cancer cells [136, 137].

To conclude this section, it seems important to report the recent discovery from Jackobsen et al. [138] which found that, as an early response, in SW620 colon carcinoma cells, DHA induced the expression of different factors involved in ER stress response, such as XBP1, PERK, ATF4, ATF6, and phosphorylated EIF2α. Worth noting, however, DHA, differently from CLA [see above and ref. 54], failed to induce apoptosis in their model. However, our group as well as several other authors [13–15, 121, 132] had previously demonstrated the ability of DHA to exert pro-apoptotic effects in different colon cancer cells. For this reason we think that additional work should be undertaken to definitely establish if apoptosis may follow, at least in some colon cancer cell types, a DHA-induced ER stress pathway, as demonstrated for CLA. Generally speaking, whereas plenty of studies have investigated the effect of saturated and monounsaturated fatty acids on ER-stress and ER-stress induced apoptosis [139–141], the possible influence of n-3 PUFAs, but also of other PUFAs, on these processes is a quite unexplored issue, worth to be further investigated in cancer and normal cells.

Other cancer cells

The pro-apoptotic effect of n-3 PUFAs has been observed in a number of other types of cancer cells [12, 142–145], and different molecular mechanisms have been proposed. Factors involved in cellular uptake and incorporation of EPA into lipids of leukemia cells have been considered essential for its apoptosis-inducing effect [142–145]. Moreover, to explain the pro-apoptotic effect of DHA in different lung, pancreatic and prostate cancer cell lines [12, 145, 146] the modification in the activity and expression of different intracellular signaling molecules have been invoked. DHA can promote apoptosis: (1) in lung cancer cells through the up-regulation of MKP-1 phosphatase and down-regulation of p-ERK1/2 and p-p38 expression [12], (2) in prostate cancer cells through the activation of PPARγ and up-regulation of SDC-1 [146], and (3) in human pancreatic cancer cells through the induction of an active extrusion process of GSH leading to its intracellular depletion and oxidative stress [145].

An effort should be certainly done to clarify how such small and simple molecules as n-3 PUFAs may exert such a pleiotropic action activating a variety of molecular pathways. It would be essential to try to identify a possible unifying hypothesis for n-3 PUFAs molecular actions, and efforts in this sense have been also previously made [147]. This subject was comprehensively treated by us in a recent report [6] and it is beyond the aim of the present review. We hypothesized the membrane enrichment in n-3 PUFAs as the initial important step to explain further molecular modifications in the cell such as: (a) the alteration in lipid peroxidation and cellular oxidative stress; (b) the alteration in membrane fluidity, structure and functions, (c) the alteration in the levels and quality of PUFA derivatives produced by their metabolism (AA and EPA or DHA derived eicosanoids and docosanoids).

Finally to conclude this section, it is important to underline the possible relevance in vivo of the above reported results obtained in vitro. The basal levels of plasma EPA and DHA in humans is approximately 20 and 80 μM, respectively [148], and marked increases (in the range of 100–250% and 25–40%, respectively) have been observed after dietary EPA + DHA (1.9 and 0.9 g/day, respectively) or fish oil supplementations (3.0 g/day EPA + DHA) [149, 150]. Also when EPA and DHA are provided by cooked salmon (1.2 g/day EPA + DHA), a similar increase is observed (129% EPA and 25% DHA). This means that, whatever is their source, EPA and DHA may easily reach in plasma those concentrations (30–100 μM) shown to be mainly effective in inhibiting cell growth and inducing apoptosis in cancer cells in vitro. These observations are in agreement with the observed anti-tumoral and pro-apoptotic effects of n-3 PUFAs observed in different animal models of cancer (see below), and suggest that these fatty acids may be considered potential chemopreventive and chemotherapic agents.

In vivo studies: animal models of cancer

The pro-apoptotic effect of dietary n-3 PUFAs was observed also in vivo using a variety of animal models of

cancer, such as rats bearing carcinogen-induced neoplastic and pre-neoplastic lesions in urothelium [151] and colon [152, 153], rat tumors transplanted in syngeneic animals including 3924A Morris rat hepatocarcinoma [154, 155], or tumors obtained implanting human cells in nude mice, such as human colon adenocarcinoma cells [15], thymoma cells [156], human breast cancer cells [157]. Moreover, increased apoptosis was observed also in normal splenic lymphocytes obtained from Balb/c mice treated with a fish oil diet [158]. Either a decrease of Bcl-2 and an increase of FAS-ligand was observed in the lymphocytes, confirming in this case the possible induction of both the intrinsic and extrinsic apoptotic pathways by n-3 PUFAs.

Moreover, a series of in vivo works demonstrated that n-3 PUFAs affect normal colonic mucosa cells by inducing apoptosis, and this has been considered as a chemopreventive effect since this cell population is highly exposed to carcinogenic agents from the diet and is subject to elevated turnover. We observed that treating rats with EPA or DHA (1 g/kg for 2 weeks) [16] suppressed cell proliferation and increased the number of differentiating and apoptotic cells in colon mucosa, without modifications of crypt morphology or number of cells/crypt. Since n-3 PUFAs did not alter the homeostasis of normal colonic mucosa and were known for their powerful anti-tumoral action, we suggested their potential chemopreventive role. In agreement, higher levels of colonic apoptosis, conferring resistance to alkylation and oxidation-induced DNA damage, were demonstrated in rats subject to AOM-induced colon tumorigenesis and treated with dietary fish oil [159, 160]. The protective effect of n-3 PUFAs was observed at both the initiation and post-initiation stages of carcinogenesis [152, 161]. This enhanced colonocyte deletion in normal mucosa was confirmed also in humans treated for 2 years with a mixture of EPA and DHA (100 and 400 mg/day, respectively) [162] or with EPA alone for a shorter period, but at higher levels (3 months, 2 g/day) [147]. Moreover, Chapkin et al. [11] observed an enhancement of the pro-apoptotic effect exerted by DHA on colonic mucosa cells when administered in combination with butyrate, a compound present in dietary fibers. It was suggested that DHA could alter colonocyte mitochondrial membrane composition, and create a permissive environment for apoptosis induced by luminal metabolites, such as butyrate [11, 133, 163]. Recently a similar apoptosis enhancing effect was observed in normal colonocytes of AOM-treated rats supplemented with both n-3 PUFAs and pectin [164]. All these findings appear to be relevant, since they support the hypothesis that the concomitant dietary intake of fibers and fish or n-3 PUFAs, at appropriate levels, may exert a strong chemopreventive action against colon cancer, and demonstrate that this protective action is based on the induction of apoptosis in colonocytes subject to carcinogenic agents.

Apoptotic effects of n-3 PUFAs in combination with conventional chemotherapeutic agents

The pro-apoptotic effect of n-3 PUFAs was also observed when they were used in combination with several drugs conventionally used in oncologic therapy [14, 165–174], and often a synergistic effect has been reported. However, the description of these chemotherapic approaches is beyond the scope of the present review, and, nevertheless, they were recently described in detail [175]. Among the drugs, whose pro-apoptotic action was enhanced by EPA or DHA there were 5-FU [14, 165], TNF-related apoptosis-inducing ligand (TRAIL) [166], AS_2O_3 [169, 170, 172, 173], Propofol [167], Imatinib [171], Celecoxib [168], Clioquinol [174], Genistein [176] and the angiogenesis inhibitor TNP-470 [177]. Most of these reports analyzed the effects in cell lines derived from colon cancer [14, 165, 166, 173], breast cancer [167, 173, 174, 176], and leukemias/lymphomas [65, 170, 171, 174]. However, the effect was more general, as demonstrated by the fact that it was observed also in a number of other cancer cell lines, including prostatic [168, 173], ovarian [173, 174], pancreatic [173], cervical [173, 174] and skin cancer [174].

n-3 PUFAs and apoptosis in other fields of human health

Different reports have also investigated the ability of n-3 PUFAs to regulate the apoptotic process in normal cells, causing beneficial health effects [117, 178, 179]. It has been found that DHA induces apoptosis in proliferating, but not in resting human umbilical vein endothelial cells (HUVEC). Induction of apoptosis in these cells has been related to the anti-angiogenic and anti-tumoral effects of n-3 PUFAs [15, 178]. On the other hand, it has been demonstrated that pretreatment of HUVEC with DHA reduces oxidative stress and apoptosis induced by 4-hydroxynonenal (HNE) [117]. This anti-apoptotic effect elicited by DHA on oxidative-stressed endothelial cells has been considered a novel mechanism to explain the atheroprotective effects of n-3 PUFAs, since apoptosis has been regarded as a potential reaction of endothelial cells after injury of the vascular endothelium. It could be judged in positive terms also the observation that DHA induces apoptosis in cultured rat mesenteric vascular smooth muscle cells [179], since it has been suggested that apoptosis of these cells may affect the structure of blood vessels, and thus explain the blood pressure-lowering effect elicited by n-3 PUFAs in hypertension [179].

DHA has often been reported to induce an anti-apoptotic effect also in neuronal cells, and it has been generally considered protective against the development of neurodegenerative disorders. DHA has been shown to protect rat embryonic primary cortical neurones and Neuro 2A neuroblastoma cells from apoptosis induced by exposure to oligomers of amyloid peptides in vitro [180]. The authors failed to find the prevention of oxidative stress, a mechanism previously invoked to explain DHA protective effect in neuronal tissue [181, 182]. In agreement, it was observed that depletion of n-3 PUFAs activates caspases in brains of transgenic mice models of Alzheimer's disease [183, 184]. Promotion of neuronal survival was elicited by DHA also in mouse neuroblastoma Neuro 2A cells in vitro [185, 186]. Consistently, the same authors found that maintaining female pregnant rats to a diet deficient in n-3 PUFAs dramatically increased apoptosis in n-3 PUFA-deficient fetal hippocampal primary cultures under trophic factor withdrawal conditions. Furthermore, it was shown that DHA decreased apoptosis induced by the amiloid peptide Aβ42 in primary human co-cultures of neurons and glia [187]. Interestingly, this effect has been related to the production of the novel discovered DHA-derived compound neuroprotectin NPD1 [188]. Recently, Mukherjee et al. [189], exploring possible therapeutic interventions for retinal degenerative diseases, found that apoptosis of primary human retinal pigment epithelial (RPE) cells caused by the bispyridinium bisretinoid A2E can be attenuated by NPD1. This finding is really worth noting, since A2E is a byproduct of phototransduction which accumulates in RPE cells in age-related macular degeneration [190–193] and may induce oxidative stress and apoptosis. In RPE cells, Neurothophin Pigment Epithelium-Derived Factor (PEDF) was able to induce the synthesis of NDP1, which therefore appears as an endogenous pro-survival agent. Moreover, a DHA treatment potentiated PEDF-stimulated NPD1 synthesis and release, demonstrating the potential beneficial action of a treatment with this fatty acid in aging.

Conclusions

Plenty of work is available demonstrating the efficacy of different dietary PUFAs and their derivatives in the modulation of incidence and progression of several diseases in which survival and apoptosis regulation may play a primary (cancer, neurodegenerative diseases) or, nevertheless, a significant role (cardiovascular and inflammatory diseases). The possibility to dietetically interfere with molecular pathways involved in apoptosis appears an intriguing therapeutic approach. It could indicate a way to achieve in a safer way what has been so far accomplished by the use of drugs which often show severe and harmful side-effects. On this basis, it may be hypothesized that the use of PUFAs in combination with conventional drugs could result in better outcome or allow a lower drug dosage. Regarding the safety of PUFA treatments, however, further studies should be conducted both in vitro and in vivo to clearly establish the optimal PUFA dosage to achieve benefits and avoid detrimental effects. Moreover, great attention should be paid on the possibility that some purified PUFAs or PUFA derivatives may act as pro-carcinogens in some contexts and towards some tissues.

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