

# Children with High Risk Neuroblastoma: Prophylactic and Therapeutic Treatment with Docosahexaenoic Acid

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Helena Gleissman

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## Abstract

Despite intense multimodal treatment of neuroblastoma consisting of surgery, chemotherapy, radiotherapy, and stem cell rescue, long-term survival is only 50% in the high-risk group. We therefore need to improve existing treatment protocols and search for new medications.

Inflammation drives cancer growth, and targeted therapy that dampens inflammatory responses is anti-proliferative. The inducible COX-2 enzyme that converts the omega-6 fatty acid arachidonic acid (AA) to various inflammatory prostaglandins is up-regulated in neuroblastoma tissue. Non steroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin synthesis have profound growth inhibitory effects on neuroblastoma cells in preclinical models.

Omega-3 fatty acids oppose the effects of omega-6 fatty acids and have been implicated in cancer treatment and prevention. Omega-3 fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are precursors of anti-inflammatory compounds. From DHA and EPA resolvins and protectins are produced, which are potent pro-resolving lipid mediators essential for the clearance of inflammatory cells and mediators at an injured site.

This chapter will discuss the toxicity of DHA to neuroblastoma cells both *in vivo* and *in vitro* as well as discuss the effects of DHA in clinical trials of various cancers. *In vivo*, DHA is able to delay time to tumor development

H. Gleissman (✉)  
Childhood Cancer Research Unit, Q6:05, Department  
of Women's and Children's Health, Karolinska Institutet,  
S-171 76, Stockholm, Sweden  
e-mail: helena.gleissman@ki.se

and reduce tumor growth in neuroblastoma xenograft models. *In vitro*, DHA acts by inducing mitochondrial-dependent apoptosis of neuroblastoma cell lines. In addition, clinical studies show that DHA acts in synergy with chemotherapy.

In summary, this chapter shows that omega-3 fatty acids such as DHA are possible new agents for neuroblastoma prevention and treatment, and suggests that these compounds be tested in clinical trials as adjuvant therapy to chemotherapeutic drugs in children with neuroblastoma.

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## Introduction

The conventional therapy of neuroblastoma has presently a 70% success rate at best. One of the novel treatment modalities that has been explored concerns fatty acids and their metabolism. Fatty acids, once thought of as solely an energy source in our bodies, have proven to be highly active molecules. They can act as ligands in signal transduction, as transcription factors that regulate protein synthesis, and as membrane components that regulate the fluidity, permeability, and dynamics of cell membranes.

In addition, fatty acids are precursors to a wide range of different lipid mediators that regulate inflammatory responses and metabolic pathways. Most fatty acids can be synthesized in the body, but not all. The essential precursors of all omega-3 fatty acids (linolenic acid; LNA), and of all omega-6 fatty acids (linoleic acid; LA), must be obtained from the diet. Thus dietary habits, and especially intake of fat, affect the body more than just influence weight and waist circumference. Dietary habits affect the system as a whole, even down to gene-level, and both the amount of fat and the kind of fat we eat can have profound and significant effects on our health.

The overall impact of proper daily fat intake for neuroblastoma patients remains to be investigated, but the data presented here show that it can be of significance. By inhibiting the omega-6 while enhancing the omega-3 metabolic pathways,

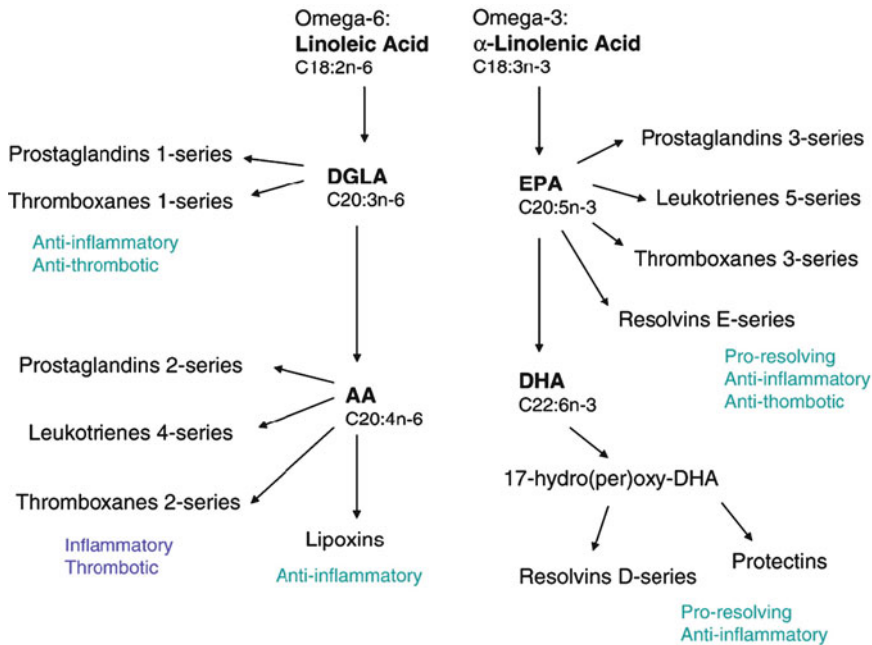
neuroblastoma growth *in vitro* and *in vivo* is reduced, as shown by *in vitro* studies on human neuroblastoma cells as well as by animal studies. In addition, this chapter proposes possible mechanisms responsible for the observed effects, and suggests how lipid mediators and enzyme inhibitors of the metabolic pathways of interest can augment the effect of cytostatic drugs.

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## Precursors and Production of Eicosanoids and Docosanoids

“Eicosa” and “docosa” are Greek words meaning 20 and 22, respectively. Eicosanoids and docosanoids are small and short-lived hormone-like molecules formed from fatty acids that regulate numerous processes in the body. They consist of 20 or 22 carbon backbones with various side chains and differently positioned double bonds. The main groups of eicosanoids are prostaglandins (PGs), thromboxanes (TXs), and lipoxins, and the main groups of docosanoids are resolvins and protectins.

The precursors of all eicosanoids and docosanoids are the following polyunsaturated fatty acids (PUFAs): Arachidonic acid (20:4, n-6, AA), Eicosapentaenoic acid (20:5, n-3, EPA), and Docosahexaenoic acid (22:6, n-3, DHA). These fatty acids are in turn the result of desaturation of LA and LNA by  $\Delta 5$ - and  $\Delta 6$ -desaturases, and of elongation by elongases. The omega-6 and the omega-3 fatty acids compete for the same desaturases and elongases, but the omega-3 family members are the preferred substrates. However, because the conversion from LA and LNA is low, PUFAs are best obtained from the diet. Once consumed, they are incorporated into phospholipids of cell membranes and the distribution is tissue-dependent. For example, the nervous system, retina, and testes are especially enriched in DHA, while most other tissues have a surplus of AA. When needed, these fatty acids are released from the cell membrane by the enzyme phospholipase A2 (PLA2). The PLA2-activity is tightly regulated by  $\text{Ca}^{2+}$  and phosphorylation, and is increased in response to factors such as



**Fig. 8.1** The wide range of compounds formed from metabolism of omega-3 and omega-6 fatty acids and their general effects. Fatty acids give rise to an enormous range of compounds with various effects. The omega-3 and omega-6 fatty acids cannot be synthesized in the human body but must be obtained from the diet. Hence,

the balance of the different eicosanoids and docosanoids and the microenvironment that they form are mainly due to dietary habits. Abbreviations: *DGLA* dihomo- $\gamma$ -linolenic acid, *AA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

inflammatory stimuli. The free fatty acids are then available for conversion to a panel of different lipid mediators by the enzymes cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 monooxygenase.

AA is converted by COX to PGs of the 2-series and to TXs (collectively termed prostanoids). It can also be converted by LOX to leukotrienes (LTs) of the 4-series, lipoxins, hydroperoxy eicosatetraenoic acids (HpETEs), hydroxyl eicosatetraenoic acids (HETEs), and hepoxilins. Furthermore, AA is converted by cytochrome 450 or cytochrome 450-induced radical oxygen species (ROS) to epoxygenase products (EETs), AA  $\omega/\omega-1$  hydroxylase products (HETEs), LOX-like products (HETEs), and primary free radical oxidation products (HpETEs) (Biondo et al. 2008). EPA is converted by COX to PGs of the 3-series, and by LOX to LTs of the 5-series, and to lipoxins. LOX can also convert both EPA and DHA

to resolvins of the E- and D-series, respectively. Furthermore, LOX can convert DHA to protectins, which will be discussed more in detail below. See Fig. 8.1 for a summary of the metabolic pathways discussed so far.

## Eicosanoid Signaling and Biological Effects

Generally, eicosanoids that are formed from the omega-6 fatty acid AA are pro-inflammatory, while eicosanoids and docosanoids formed from the omega-3 fatty acids DHA and EPA are anti-inflammatory. The reality is not as simple as stated here, however; the anti-inflammatory lipoxins that are formed from AA are a good example of an exception to this rule. The role of eicosanoids as inducers and regulators of inflammatory response has been thoroughly

studied for decades. Recently, the docosanoids have been studied more extensively, and it has become evident that resolution of inflammation, a process previously thought to occur passively, is governed by the production of docosanoids—that is, the resolvins (Serhan et al. 2008).

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## Docosahexaenoic Acid (DHA)

The one essential omega-3 fatty acid is LNA, from which both EPA and DHA are formed. EPA and DHA contain different numbers of double bonds, but the first double bond from the methyl end of the carbon chain is always situated between carbons number three and four. LNA can primarily be found in leafy green vegetables, walnuts, and canola oil. By the enzymes  $\Delta$ -6-desaturase,  $\Delta$ -5-desaturase and elongase, LNA is converted to EPA and DHA. However, this conversion only takes place to a limited extent, and occurs more in women than in men. The primary source of EPA and DHA for humans is fatty fish such as salmon, herring, and mackerel. These fish are rich in omega-3 fatty acids because they consume photosynthetic and heterotrophic microalgae of the genus *Schizochytrium* that produce EPA and DHA, which become increasingly concentrated in organisms as they move up the food chain.

DHA (*all-cis*-docosa-4,7,10,13,16,19-hexaenoic acid) has a chain length of 22 carbons that contains six double bonds, which makes it the longest chain and most unsaturated fatty acid commonly found in biological systems. In the human body, it is either acquired from the diet or it is derived from EPA via docosapentaenoic acid (DPA) as an intermediate; a pathway known as Sprecher's shunt. In humans, DHA is especially enriched in neural tissue. It comprises 40% of the PUFAs in the brain, 60% of the PUFA in the retina, and 50% of the weight of the neurons' plasma membrane. It is esterified into phospholipids embedded in cell membranes, especially of phosphatidylethanolamine (PE) and phosphatidylserine (PS), preferably in sn-2 position (Piomelli et al. 2007).

In membranes containing DHA, the packing is distorted by steric restrictions associated with the presence of multiple rigid double bonds; that is,

the bent shape of this fatty acid prevents a perfect fit in the membrane. This is thought to significantly alter many basic membrane properties including acyl chain order and fluidity, phase behavior, elastic compressibility, permeability, fusion, flip-flop, and protein activity (Stillwell and Wassall 2003). Once taken up by cells or released from the cell membrane, DHA acts as a ligand to certain nuclear receptors, such as the PPAR- $\gamma$  (Gani and Sylte 2008) and the RXR receptor (Lengqvist et al. 2004). In conjunction with these receptors that act as transcription factors, DHA helps regulate various biological functions ranging from lipid metabolism and homeostasis to cell differentiation and cell death (Berquin et al. 2008). Many of the receptor-mediated effects of DHA are still unexplored.

In addition, DHA has been shown to influence signal transduction of a variety of pathways. For example, DHA activates the Jak/Stat pathway; downregulates protein kinase C, Ras, ERK and NF- $\kappa$ B; sustains phosphorylation of EGFR; and influences the Bcl-2 family of proteins regulating cell growth (Berquin et al. 2008). Furthermore, DHA can modulate the translation machinery by reducing intracellular Ca<sup>2+</sup> stores (Jude et al. 2006).

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## Areas of Application of Docosahexaenoic Acid

In 1970 the pioneers of omega-3 fatty acid research, Dr. Dyerberg and Dr. Bang from Denmark, visited Greenland on an expedition to understand how the Inuits could eat a high-fat diet and still have one of the lowest death rates from cardiovascular disease in the world. Their discovery that the Inuits had favorable blood lipids resulted in a publication in *Lancet* in 1971 (Bang et al. 1971). Not until some years later had Dr. Dyerberg and Dr. Bang analyzed all blood samples on an old gas chromatogram and found two fatty acids, DHA and EPA. This was the birth of omega-3 fatty acid research. Since then, mainly through dietary studies, DHA has been associated in beneficial ways with an enormous range of human afflictions including cancer, heart disease, rheumatoid arthritis, asthma, lupus, alcoholism, visual acuity, kidney

disease, respiratory disease, peroxisomal disorders (Zellweger's Syndrome), dermatitis, psoriasis, cystic fibrosis, schizophrenia, depression, neurologic and brain development, malaria, multiple sclerosis, and even migraine headaches. In fact, it is difficult to find any human disorder where omega-3 fatty acids have not been tested.

The common denominator that might explain the beneficial effects of omega-3s and DHA in particular in this great variety of diseases and symptoms is its anti-inflammatory properties. Until recently, it was unknown how DHA exerted these anti-inflammatory effects. One postulated reason is that DHA replaces AA in cellular membranes, and hence less AA is available for conversion by COX and LOX to pro-inflammatory eicosanoids. Furthermore, DHA competes with AA for binding sites on the COX enzyme, and is actually the preferred substrate. These indirect mechanisms for inhibiting inflammatory responses seem reasonable and have proven to be correct, but a huge step was taken towards understanding DHA's beneficial effects when resolvins and protectins were identified Serhan et al. (2002).

Resolvins of the D-series are produced from DHA by the enzymes 5-LOX and 15-LOX, or by a COX-enzyme that has been acetylated by aspirin. Protectins are also formed via LOX-mediated pathways. These lipid mediators, and also resolvins of the E-series (EPA-derived), powerfully clear inflammation by clearing neutrophils and macrophages from inflammatory sites. Actually, they are essential for resolution of an inflammatory response, a process that was formerly believed to occur passively (Serhan et al. 2008). These newly discovered substances are currently in clinical testing (as reported by Resolvyx Pharmaceuticals at [www.resolvyx.com](http://www.resolvyx.com)). However, many of the mechanisms behind the positive effects observed by DHA and EPA are still elusive.

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## **Docosahexaenoic Acid in Cancer Prevention**

What we know about the role of omega-3 fatty acids in cancer development is almost exclusively based on epidemiological observations. There are

many challenges in interpreting data of this sort due to heterogeneity in study design and the fact that subjective dietary questionnaires often are used instead of biomarkers. However, a small to moderate reduction in cancer risk, or no effect, is most often the result of such studies.

An observational study that supports the theory of cancer prevention in children by omega-3 fatty acids was carried out among the native Inuit population of Alaska (Lanier et al. 2003). In this study, childhood cancer incidence was analyzed from 1969 to 1996, and apart from the increased number of hepatocellular carcinomas in this population due to Hepatitis B infection (a phenomenon that disappeared after initiation of a vaccination program), the rate of childhood cancer was significantly lower compared to a North American population. Specifically, the incidence of neuroblastoma was reduced ten-fold (0.9/million vs 7.9/million). The Inuit population of Alaska pursue a lifestyle where fish and seal meat are the main commodities, and their DHA levels are several-fold higher than in Caucasians, where the omega-3/omega-6 ratio has dropped dramatically over the past decades (Simopoulos 2006).

In the Japanese population, whose traditional diet includes much fish, the incidence of certain cancers such as breast cancer has increased along with a more "westernized" food consumption and lifestyle. Since this observation was made, several studies have pointed out that omega-3 fatty acid consumption is associated with decreased cancer risk of the breast, prostate, colon and kidneys as summarized by (Berquin et al. 2008).

There is a large, randomized, double-blind and placebo-controlled study ongoing called VITAL (VITaminD and Omega-3Trial (Manson et al. 2011). This study aims to investigate DHA, EPA (Omacor® fish oil, 1 g/day), and Vitamin D (cholecalciferol, 2,000 IU/day) in the primary prevention of cancer and cardiovascular disease among 20,000 US citizens aged over 50 years. The treatment period will be 5 years. The study is one of the first and largest studies where cancer prevention will be studied by intervention and not by observation only. We await the results with anticipation.

Animal studies on DHA supplementation as cancer prevention have shown that a DHA-enriched or fish oil-enriched diet can inhibit the formation of not only neuroblastoma (Gleissman et al. 2010b; Barnes et al. 2012), but also papillomas, mammary carcinogenesis, carcinogenesis of the large and small intestine, and carcinogenesis of the lung. DHA-enriched diets can also reduce formation of aberrant crypt foci, metastatic colon cancer carcinoma, sarcoma, and prostate cancer.

The Fat-1 transgenic mouse model provides strong evidence that DHA and DHA-derived compounds may have significance in cancer development (Kang et al. 2004). These mice carry a gene which encodes a desaturase that catalyzes conversion of omega-6 to omega-3 fatty acids, a feature that is lacking in most mammals, including humans. In this mouse model where the omega-3/omega-6 ratio is increased, melanoma formation and growth, colitis-associated colon cancer growth, prostate cancer growth and breast cancer growth were all reduced compared to tumor growth in non-transgenic animals.

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## Docosahexaenoic Acid in Cancer Therapy

For obvious ethic reasons, DHA is never given as single therapy to humans with a tumor burden, but is combined with adequate cytostatic drugs. The use of DHA as an adjuvant to conventional therapy has proved to be efficient. DHA can potentiate the anticancer effects of both chemo- and radiotherapy. (The mechanisms behind the observed synergistic effects are discussed in the next section.)

One therapeutic study in breast cancer patients where DHA was combined with epirubicine, cyclophosphamide, and 5-fluorouracil emphasizes that an inter-individual uptake and incorporation of DHA alters the treatment response (Bougnoux et al. 2009). Patients were supplemented with DHA daily during the chemotherapy cycles and could then be divided into high and low incorporating groups based on the DHA levels in plasma and red blood cells. The high

incorporating group was characterized by longer overall survival and delayed time to tumor progression compared to the low incorporating group.

In another study, non small cell lung cancer (NSCLC) patients were given DHA and EPA together with their first-line therapy (platinum-based regimens such as carboplatin in combination with vinorelbine or gemcitabine). Patients in the supplemented group had an increased response rate, greater clinical benefit and greater 1-year survival compared with the control group (Murphy et al. 2011).

In some settings, supplementation with DHA may not alter treatment response, but can still be beneficial since it improves the nutritional status and quality of life of patients. DHA sometimes even reduces unwanted side-effects of conventional treatment. In a study on NSCLC patients the supplemented group reported significantly higher on the quality of life parameters, physical and cognitive function, global health status and social function than the control group. The intervention group showed a higher Karnofsky Performance Status and tended to have a higher physical activity (van der Meij et al. 2012).

The aim of another study on patients with lung cancer was to investigate the effect of EPA and DHA on inflammatory condition, and oxidative and nutritional status. A significant increase of body weight in the supplemented group was observed. Levels of inflammatory biomarkers differed significantly between the supplemented and placebo groups and progressively decreased during chemotherapy in the supplemented group, evidencing anti-inflammatory action. Concerning oxidative status, plasma reactive oxygen species levels increased in the placebo group. These anti-inflammatory and anti-oxidative actions could be considered a preliminary goal in anti-cachectic therapy, as cachexia is a common problem among cancer patients (Finocchiaro et al. 2011).

There are currently more than 30 ongoing clinical trials in the USA where DHA or omega-3s are being tested for cancer prevention, support, or therapy (as reported by National Cancer Institute at <http://www.cancer.gov/clinicaltrials>). As single therapy, DHA has been given to animals



in three different studies. In two, the animals were xenografted with SK-N-BE(2) or SK-N-SH neuroblastoma cells (Gleissman et al. 2010b; Barnes et al. 2012), and in the other with BxPC-3 pancreatic cancer cells. In the latter study, DHA was also combined with curcumin, a dual COX-2 and 5-LOX inhibitor. Tumor growth was inhibited by DHA as single therapy, and the inhibitory effect increased with the combination of DHA and curcumin. DHA as single therapy does have effect on tumor burden, but is not efficient enough to be used as such.

## Mechanisms of Action of Docosahexaenoic Acid in Cancer Cells

### Apoptosis/Autophagy

DHA induces dose-dependent apoptosis of cancer cells (Lindskog et al. 2006; Gleissman et al. 2009). Serini et al. (2009) have reviewed several suggestions of mechanisms that seek to explain this phenomenon, including both the intrinsic and the extrinsic pathways. DHA modifies the expression of proteins of the Bcl-2 family by increasing the levels of the pro-apoptotic proteins Bak and Bcl-xS and reducing those of the anti-apoptotic proteins Bcl-2 and Bcl-xL (Manna et al. 2008). DHA induces cytochrome *c* release from mitochondria and mitochondrial membrane depolarization. DHA causes downregulation of Wnt/Beta-catenin signalling and inhibits syndecan-1 of the MEK-Erk pathway. Furthermore, DHA induces autophagy through p53/AMPK/mTOR signalling.

### Oxidative Stress

The initial event in the oxidative metabolization of all PUFAs is abstraction of hydrogen. This occurs at an increased rate when the internal redox balance of a cell is seriously disturbed and the production of initiators, such as radical oxygen species (ROS), cannot be sufficiently suppressed, a common scenario in tumor cells. The most common ROS are hydroxyl radicals, superoxide radicals,

alkoxyl radicals, peroxy radicals, singlet oxygen, ozone, anions, and hydrogen peroxide.

Intracellular accumulation of ROS leads to disruption of the mitochondrial membrane potential, to release of cytochrome *c* with consecutive activation of the caspase cascade, and, ultimately, to programmed cell death through apoptosis as discussed above. The glutathione (GSH) system (GSSG/2GSH) is considered to play a central role in maintaining cellular redox balance by scavenging radicals formed by oxidation. Since DHA is a highly unsaturated PUFA, it is susceptible to peroxidation and can cause accumulation of a surplus of ROS that cannot be scavenged by the cancer cells. Addition of anti-oxidants to cells incubated with DHA diminishes the toxic effects, strengthening this theory (Lindskog et al. 2006).

### Potential of Cytostatic Drugs

The effect of combined treatment of DHA with cytotoxic drugs or radiation seems to be a potential way to clinically apply DHA in cancer treatment. DHA in combination with doxorubicin, irinotecan, cisplatin, melphalan and vincristine on neuroblastoma cell survival shows additive or synergistic interactions (Lindskog et al. 2006). A few different mechanisms whereby DHA enhances the effects of chemotherapeutic drugs have been suggested and summarized (Biondo et al. 2008; Siddiqui et al. 2011) and include:

- DHA acts on membrane-associated signal transduction, such as decreased Ras-, PI3K/AKT- and Her-2/neu- signaling, and changes lipid raft composition
- DHA-peroxidation stimulates formation of oxygen free radicals
- DHA inhibits chemotherapy-induced NF- $\kappa$ B activation
- DHA enhances drug uptake by altering membrane properties and decreasing production of MDR proteins
- DHA induces apoptosis by modulating the effects of pro- and anti-apoptotic proteins in the Bcl-2 family of proteins
- DHA affects several other intracellular targets including cyclooxygenase-2, peroxisome

proliferator-activated receptor gamma, mitogen-activated protein kinase, and AKT.

### **Inhibition of COX-2 and PGE<sub>2</sub>**

DHA is incorporated into cell membranes at the expense of AA, which leads to less formation of AA-derived PGE<sub>2</sub>, a lipid mediator that has been shown to drive tumor growth. Several studies have demonstrated that supplementation of omega-3 PUFA to cells, animals, and humans reduces the AA-derived eicosanoids.

COX-2 and microsomal PGE synthase 1 (mPGES-1), the enzymes responsible for converting AA to PGE<sub>2</sub>, are highly expressed in neural tumors and inhibition of these enzymes has profound effects on the survival of these tumors (Baryawno et al. 2008). The effect of combining DHA with the COX-2 specific inhibitor celecoxib in neuroblastoma cells and other various human cell lines show that these compounds induce synergistic cytotoxicity. Mechanisms seem to be both COX-2-dependent, such as blockage of COX-2 and inhibition of AA metabolism, and COX-2 independent, such as induction of heat shock proteins and modulation of NF-κB activity and steroid receptors. In addition, DHA may also induce cytotoxicity by binding to catalytic sites of elongases and desaturases (Larsson et al. 2004).

### **Cytotoxic Intermediates of DHA Metabolism**

When DHA is converted to resolvins and protectins, two specific intermediates are formed, namely 17-hydroperoxy-DHA (17-HpDHA) and 17-hydroxy-DHA (17-HDHA). When comparing DHA, 17-HpDHA and 17-HDHA with respect to tumor cell toxicity, 17-HpDHA displays the highest cytotoxic potency. Furthermore, DHA and 17-HpDHA, but not 17-DHA, induces apoptosis in neuroblastoma cells (Gleissman et al. 2009). Hence, DHA probably has the capacity to induce cytotoxicity in neuroblastoma cells by the

intracellular formation of hydroperoxy fatty acids as described above and as hypothesized by Siddiqui et al. (2008) in a review on DHA's many oxidation products.

If a non-stereospecific DHA-derived hydroperoxy fatty acid is exogenously supplied to neuroblastoma cells it causes apoptosis through several apoptotic hallmarks including nuclei condensation, DNA fragmentation, poly-(ADP-ribose) polymerase cleavage, and increased activity of caspase-3. In addition hydroperoxy fatty acids cause release of cytochrome *c*, increased Bcl-2 expression, and attenuation of mitochondrial membrane potential. These data indicate that DHA hydroperoxides induce apoptosis in human neuroblastoma cells, which is mediated by the mitochondrial (intrinsic) pathway.

### **Adhesion and Angiogenesis**

Two of the first steps required for tumor establishment are adhesion and angiogenesis. DHA can inhibit adhesion, probably by down-regulating Rho GTPase, inhibiting cytoskeleton reorganization, and reducing ICAM-1 and VCAM-1 protein expression. DHA has also been shown to decrease TNFα-induced monocyte rolling, adhesion, and transmigration. These effects might also be applicable to tumor cells in the process of adhering to tissue sites. Furthermore, DHA has been shown to reduce angiogenesis, probably by decreasing levels of vascular endothelial growth factor, platelet-derived growth factor, and platelet-derived endothelial cell growth factor (Victory et al. 2007).

### **Metastatic Spread**

In a laboratory setting, DHA inhibits invasion through matrigel of both urinary bladder and pancreatic cancer cells. It does so by down-modulation of Granzyme B, a serine proteinase with extracellular functions that promote invasion (D'Eliseo et al. 2011).



In a mouse model of human breast cancer cell metastasis to bone, it has been shown that a diet enriched in DHA and EPA prevents the formation of osteolytic lesions in bone, indicating suppression of cancer cell metastasis to bone. DHA and EPA also significantly attenuate the migration/invasion of breast cancer cells in culture. One proposed mechanism is that DHA and EPA significantly inhibit the expression of CD44 protein and mRNA by a transcriptional mechanism. Aberrant increased expression of CD44 is associated with generation of cancer stem cells, which contribute to metastasis of breast cancer cells. Furthermore, in the mice fed with a fish oil diet, the levels of CD44 mRNA and protein in the tumors were reduced (Mandal et al. 2010).

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### **Can Docosahexaenoic Acid Be Given to Children with Neuroblastoma?**

The information on pharmacokinetics of DHA in children is limited. However, DHA supplementation to children has been done in different studies with doses up to several grams per day, resulting in increased plasma levels and without much adverse side effects. Some studies report but a few gastrointestinal problems such as diarrhea after high doses of DHA. This problem is adjustable by dose-titration. The fear of bleeding due to DHA-supplementation seems to be uncalled-for. DHA does increase bleeding time, but not to an extent that has any clinical implication. DHA supplementation effectively alters plasma lipid composition in children, an important prerequisite for any attempt to validate these experimental findings in children with neuroblastoma (Lien 2009).

In addition to being toxic to cancer cells, DHA can protect healthy nervous tissue via the downstream products protectins (Farooqui 2012). This is of particular interest when treating cancers of the nervous system such as neuroblastoma and also medulloblastoma, another childhood cancer of the nervous system with poor outcome. In this case, DHA may work as both a sword and a

shield (Gleissman et al. 2010a), which would be highly useful since treatment often gives severe sequelae.

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### **Summary**

DHA is a safe compound that exerts anti-proliferative effects on cancer cells and works in synergy with chemotherapeutic drugs. It is easy to administer and monitor. We therefore suggest that DHA should be evaluated as an adjuvant to chemotherapy during treatment of neuroblastoma in clinical trials. In some settings, DHA may increase treatment efficacy by increased tumor cell killing and prolonged survival of patients. DHA may also reverse adverse side effects and malnutrition, which could allow intensified or prolonged treatment if necessary. In other settings DHA may be inert, but as far as studies have shown it will not counteract current conventional treatment.

Epidemiological observations show that DHA-intake may reduce neuroblastoma incidence as well as other cancer forms. This is of high interest when it comes to nutrition in early childhood and pregnant women. Because DHA acts anti-inflammatory and pro-resolving via resolvins, the aspect of cancer-associated inflammation is highly interesting. Other anti-inflammatory agents used in cancer treatment, such as COX-2 inhibitors that have been reported to increase the risk of severe side-effects, might be replaced by these newly identified lipid mediators. Resolvins work as agonists of resolution instead of antagonists of inflammation, which indicates that less toxic side effects due to inhibition of prostaglandin and thromboxane synthesis can be expected.

In conclusion, we are beginning to understand the physiology and molecular basis of omega-3 fatty acids and thereby gradually encompass the full clinical potential of these compounds. Hopefully, this understanding can quickly pass on into the clinic, and possibly save lives and enhance quality of life for numerous neuroblastoma patients.

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