Docosahexaenoic fatty acid (DHA) in the regulation of colon cell growth and cell death: A review

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Background. Experimental, epidemiological and clinical data substantiate the beneficial role of n-3 polyunsaturated fatty acids (PUFAs) in preventing inflammation and cancer of the colon. This review covers the unsaturated docosahexaenoic fatty acid (DHA), describes some of its important cellular and molecular mechanisms, its interaction with another dietary lipid, butyrate and with endogenous apoptotic regulators of the tumour necrosis factor (TNF) family. We also discuss the clinical impact of this knowledge and the use of these lipids in colon cancer prevention and treatment.

Results. From the literature, DHA has been shown to suppress the growth, induce apoptosis in colon cancer cells *in vitro* and decrease the incidence and growth of experimental tumours *in vivo*. Based on these data and our own experimental results, we describe and discuss the possible mechanisms of DHA anticancer effects at various levels of cell organization. We show that DHA can sensitize colon cancer cells to other chemotherapeutic/chemopreventive agents and affect the action of physiological apoptotic regulators of the TNF family.

Conclusion. Use of n-3 PUFAs could be a relatively non-toxic form of supportive therapy for improving colon cancer treatment and slowing down or preventing its recurrence. However, it is necessary to use them with caution, based on solid scientific evidence of their mechanisms of action from the molecular to the cellular and organism levels.

Key words: polyunsaturated fatty acids, docosahexaenoic acid, colon cancer, apoptosis, cell signalling, anticancer therapy

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INTRODUCTION

Essential polyunsaturated fatty acids (PUFAs) are important biocompounds with structural and functional roles in both normal and malignant cells¹. They are divided into two main types: the n-6 series, derived from linoleic acid (LA, 18:2, n-6), e.g. gamma-linolenic acid (GLA, 20:3) and arachidonic acid (AA, 20:4) present in plant oils, and the n-3 series, derived from alpha-linolenic acid (ALA, 18:3, n-3), e.g. eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) present mainly in algal and fish oils^{2,3}. Since mammals are deficient in the *fat-1* gene encoding n-3 desaturase⁴, the availability of PUFAs depends on external supply.

PUFAs play a vital role at all levels of the organism⁵. It has also been shown that the composition of the PUFAs, particularly the ratio of n-3 to n-6 type in the diet, is of key importance⁶. PUFAs influence many physiological functions, including modulation of cell structure and behaviour in all stages of carcinogenesis and metastasis. During the last 25 years, hundreds of papers describing the effects of PUFAs on normal and cancer cells, differences between n-6 and n-3 PUFAs, and proposed mechanisms of their action on molecular, cellular and organismic levels have been published. Despite much contradiction in the literature, there is a consensus that high calorie and fat intake are risk factors for colon cancer and that n-6 PUFAs

(from plant oils rich in LA) positively correlate with intestinal tumorigenesis. On the other hand, PUFAs of the n-3 series, especially EPA and DHA (rich in fish oil), are shown to be protective against cancer development^{7,8}. The anti-inflammatory and anti-cancer effects of n-3 PUFAs and fish oil have been widely documented experimentally using *in vitro* as well as *in vivo* systems and clinical studies^{9,10}. Nutritional supplementation with n-3 PUFAs either as enteral or parenteral nutrition with lipid emulsions can improve nutritional variables, cancer cachexia, immunity, clinical outcomes, and postoperative complications^{11,12}. Moreover, nutritionally induced changes in fatty acid composition may result in increased sensitivity to chemo- and radiotherapy and decreased side effects¹³.

The aim of this review is to summarize knowledge of 1) the beneficial effects of n-3 DHA in colonic tissue, 2) describe potential cellular and molecular mechanisms of its action, 3) highlight DHA ability to sensitize cancer cells to other anticancer agents and physiological cell growth and apoptosis regulators, and 4) discuss the clinical significance of the findings.

Synthesis and metabolism of DHA

The n-3 high double bonded PUFAs, DHA and EPA, are physiologically very important compounds. Since humans cannot effectively metabolize ALA, algal and fish oils remain their main sources¹⁴. DHA from the diet is

obviously introduced into the human body in the form of triglycerides (TGs). Dietary TGs undergo enzymatic hydrolysis in the upper intestine into free fatty acids and 2-monoglycerides. After diffusion through the epithelium, they are incorporated into new or reconstituted TGs and, in the form of chylomicrons, they enter the lymph for transport to blood and incorporation into plasma lipids, erythrocyte membranes, platelets, adipose and other tissues. In the tissues, free fatty acids are hydrolyzed for metabolism or for cellular uptake. They can be stored in the cells as an energy source or serve as structural components of cell membranes¹⁵. There is also a limited capacity of the liver to synthesize DHA from ALA or EPA by desaturation and elongation to a 24:6 product with subsequent mitochondrial beta oxidation¹⁶. The liver-derived DHA reaches the brain and other organs through the circulation, probably bound to proteins that are also synthesized by hepatocytes¹⁷. DHA can undergo mitochondrial beta oxidation or can be metabolized via carnitine-independent peroxisomal beta oxidation to shorter-chain fatty acids. The new products may undergo further peroxisomal degradation or transport into the mitochondria for oxidative metabolism.

The dietary form of ingested DHA markedly affects its kinetics and partly its metabolic fate¹⁸. DHA is better absorbed in lipid mixture which is pre-emulsified before ingestion¹⁹. The reported DHA turnover rate is about 20 hours after introduction into the human body, and there is a difference in DHA uptake depending on its enrichment in low-density lipoprotein (LDL) or high-density lipoprotein (HDL). DHA circulation as LDL results in a longer body turnover and a more efficient uptake by different organs²⁰. There are some reports showing that the DHA status can be improved by long-term intake of vegetable oils containing ALA and less LA (ref.²¹).

DHA is effectively incorporated into cellular TGs, cholesterol esters, and various classes of plasma membrane and mitochondrial phospholipids (PLs) (ref.²²). In response to elevated fatty acids, lipid droplets (LDs) containing mostly TGs are rapidly formed in the cytoplasm of cultured cells as well as under *in vivo* conditions. LDs appear to be complex, metabolically active organelles directly involved in membrane traffic and PL recycling. LDs serve as intracellular deposits of PUFAs, reservoirs for various lipid metabolism enzymes, and as a site for lipid mediator synthesis²³.

Due to its unique physical and biological properties, DHA influences membrane structure and dynamics, acyl chain flexibility, formation of lateral domains, increases its permeability to water, but interacts poorly with cholesterol. These characteristics mean that DHA can modulate the structure of specific membrane microdomains (lipid rafts and caveolae) and cell membrane functions. This includes the activity of membrane transporters, receptors and associated signalling pathways, and consequently affects cell behaviour²⁴.

Dietary intake and health benefits

A large number of research results show that nutritional lipid composition has the potential to prevent and

treat a number of diseases, including cancer. Since the Industrial Revolution, humans in the West have fundamentally changed their dietary habits towards increased consumption of animal protein and animal fat, decreased consumption of antioxidants, and increased n-6:n-3 fatty acid ratio²⁵. The high fat and calorie diet together with excessive amounts of n-6 PUFAs and a high n-6/n-3 ratio, found in Western diets, promote the pathogenesis of a range of diseases from cardiovascular disease to obesity, diabetes, autoimmune disease, inflammation, and cancer²⁶. It has been reported that DHA in particular can reduce these disorders^{27, 28}. In addition, DHA is the predominant structural fatty acid in human neural tissue and DHA deficits are implicated in learning and cognitive dysfunction²⁹, neuropsychiatric disorders³⁰, and neurodegenerative conditions such as Alzheimer's disease³¹.

For these reasons, n-3 PUFAs, particularly DHA, are important in primary health prevention for the general population. Their intake should be in accord with the suggestions based on epidemiological studies^{32, 33}. Differences in these recommendations reflect distinct experimental approaches, characterization and evaluation of experimental groups. For example, in 1999 the International Society for Study of Fatty Acids and Lipids (ISSFAL) recommended 0.22 g/day for DHA and EPA, which seemed to be adequate intake³⁴. The ISSFAL updated these data for cardiovascular prevention and proposed 0.5 g/day for DHA and EPA intake. The Institute of Medicine in the United States published 0.5 g /day as an adequate intake of n-3 PUFA (IOM, 2002 (ref.³⁵)). There are also national specific recommendations for DHA and EPA intake such as the British Nutrition Foundation³⁶, which suggested 1.1 g/day of DHA and EPA for females and 1.4 g/day for males³⁶. The Health Council of the Netherlands proposed a desirable population intake of 0.45 g/day of n-3 PUFA from fish diet³⁷. In 2008, the World Health Organisation published a proposed adequate intake of DHA and EPA of 0.25 g/day³⁸. In the Czech Republic, increased consumption of sea fish and fish products (ca. 400 mg per week) and a maximal ratio of n-6/n-3 PUFAs 5:1 are recommended³⁹.

There is accumulating evidence that dietary factors, particularly n-3 PUFAs, prevent colon inflammation and carcinogenesis⁴⁰. The results mainly from *in vitro* and *in vivo* experimental systems are supported by epidemiological studies confirming that higher concentrations of n-3 PUFAs in cell membranes are associated with lower cancer risk⁴¹. The results also indicate that n-3 PUFAs have minimal negative effects on normal cells⁷. In 1996 an epidemiological study from 22 European countries, USA and Canada was published, suggesting that diets rich in n-3 PUFAs may decrease the risk of colorectal cancer (CRC) (ref.⁴²). There are also other studies describing a relation between the consumption of fish food and lower incidence of CRC in the population of Asian Indians in the U.S. (ref.⁴³), and the Middle East countries (Egypt, Israel, Cyprus, Jordan) (ref.⁴⁴). However, in one systematic review summarizing published and unpublished results on the relation between the consumption of n-3 PUFAs and cancer incidence, the authors concluded that there

was not sufficient evidence for the preventive effects of n-3 PUFAs on colon carcinogenesis⁴⁵.

Owing to inconsistencies in the experimental, clinical and epidemiological data, further studies elucidating and verifying the cellular and molecular mechanisms of n-3 PUFA action are necessary for a better understanding of their role in human cancer, reliable nutritional recommendation, and use in therapy. In the following section, we summarize mainly recent knowledge on the molecular mechanisms associated with DHA effects on colon cell growth and cell death.

MOLECULAR MECHANISMS OF DHA ACTION IN COLON CANCER CELLS

The epithelium of the mammalian intestine is a continuously renewing tissue serving numbers of critical physiological functions. Dynamic balance between cell production at the base and cell death at the surface of the colonic crypts is precisely regulated by a number of physiological endogenous factors such as hormones and cytokines. The pathogenesis of CRC is a long and multifactorial process which involves mutations in specific oncogenes and tumour suppressor genes and alterations in gene expression which are induced by epigenetic and non-genotoxic mechanisms⁴⁶. This process is significantly influenced by environmental factors and lifestyle, especially diet composition⁴⁷.

Experimental studies have shown that DHA functions as a potent suppressor of colon cancer cell proliferation *in vitro*⁴⁸ as well as *in vivo*⁴⁹, simultaneously inducing cell death in a dose- and time-dependent manner⁵⁰. The inhibitory effects of a fish oil diet or purified EPA or DHA on the incidence and number of colon preneoplastic loci or tumours induced by azoxymethane (AOM) or 1, 2-dimethylhydrazine in rats have been studied for a long time⁵¹. N-3 PUFAs, preferentially DHA, suppressed *in vivo* growth and induced apoptosis of human colon carcinoma xenografts in athymic nude mice⁵². A lower incidence and growth of AOM-induced tumours were observed in transgenic mice carrying the *fat-1* gene, which increases endogenous levels of n-3 PUFAs (ref.⁵³). Data from scarce patient interventional trials showed that n-3 PUFA supplementation (about 4–9 g per day) modified colonic mucosa and the plasma fatty acid pattern, decreased colonic or rectal epithelial cell proliferation, and induced apoptosis in subjects at risk of CRC (ref.⁵⁴). Several potential mechanisms operating at various cellular levels have to be considered in DHA antiproliferative and apoptotic effects.

DHA-induced changes of membrane properties and microdomains

The main physiological role of PUFAs, namely DHA, is associated with modulation of the structure and function of cell membranes. Ingestion of PUFAs leads to their distribution to virtually every cell in the body and influences mainly the lipid profile and fatty acid composition of plasma, nuclear and mitochondrial cell membranes, affecting their structure and fluidity, functions of mem-

brane-bound proteins, and lipid-mediated signalling⁵⁵. DHA is thought to be the essential component of lipid rafts which are small platforms of proteins and lipids in plasma membrane rich in cholesterol and sphingolipids⁵⁶. These structures contribute to the unique biophysical property of the cell membrane called liquid-ordered state, and are functionally implicated in the compartmentalization, modulation, and integration of cell signalling responsible for the regulation of cell growth, survival, and adhesion⁵⁷.

The epidermal growth factor receptor (EGFR) is an interesting example of protein localized in lipid rafts, whose signalling can be effectively modulated by DHA. In addition, targeting EGFR signalling is effective in selective elimination of cancer cells⁵⁸. In DHA-treated colon, lung or breast cancer cells, EGFR was excluded from caveolin-rich lipid raft fractions, which had a significant impact on the related kinase signalling pathways⁵⁹. Moreover, many proteins involved in signal transduction are modified by acyl moieties so that they are attached to the plasma membrane and/or concentrated in lipid rafts⁶⁰. Regulation of dynamic membrane translocation of these proteins is crucial to their function and shown to be affected by DHA. It has been demonstrated that DHA suppresses activation of Ras, a 21 kDa guanine nucleotide binding protein, by limiting its localization to the cell membrane. This result is relevant to colon cancer treatment, as a high frequency of Ras mutations has been found in human adenomas and adenocarcinomas⁶¹.

Intracellular signalling pathways affected by DHA

DHA and its metabolites are involved in the regulation of many cell signalling pathways, contributing to the activation or inhibition of various components of the molecular machinery⁶². DHA influences not only transduction of signals from the extracellular space, it also functions as a mediator and modulator of the inter- and intracellular signalling network⁶³.

DHA has been shown to actively modulate protein kinase C (PKC)-related signalling, having various context-dependent impacts on different PKC isoforms. Specifically, the expression of the PKC ϵ isoform was decreased by DHA in colon epithelial cells treated with arabinosylcytosine, and affected its cytotoxic potential⁶⁴. A significant decrease in the level of membrane-associated PKC β II, which together with increased transforming growth factor β receptor type II (TGF β RII) and cyclooxygenase 2 (COX-2) expression promotes colon carcinogenesis, was observed in the colon epithelium of animals fed with n-3 PUFAs. PKC β II, which is induced early during colon carcinogenesis, represents a new and potentially highly effective target for chemopreventive therapy in colon cancer⁶⁵.

Studies using mostly human colon adenocarcinoma cell lines *in vitro* showed that DHA inhibited cell proliferation and induced apoptosis possibly by decreasing signal transduction through the PI3K/Akt cell survival pathway and altered the levels of phosphorylated p38 and ERK1/2 kinases. The PI3K/Akt signalling pathway plays a pivotal role in the regulation of colon growth and apoptosis, and

can contribute to cancer progression⁵⁰. DHA also reduced phosphorylation of phosphatase and tensin homolog protein (PTEN), which functions as a negative regulator of the PI3K/Akt pathway⁶⁶.

The Wnt/beta-catenin pathway is another important regulator of the apoptotic process in colorectal cancer cells. Dysregulation of Wnt signalling and beta-catenin expression is believed to be central to the early stages of sporadic carcinogenesis in humans⁶⁷. It has been demonstrated that DHA reduces the expression of beta-catenin protein in Caco-2 cells, simultaneously decreasing the levels of COX-2 and inducing apoptosis⁶⁸. Long-term feeding of rats with a diet which included corn oil or beef tallow increased AOM-induced colon carcinogenesis through Wnt/beta-catenin signalling. On the other hand, a diet containing DHA decreased Wnt expression, and the beta-catenin signalling pathway, thus reducing cancer proliferation⁶⁹.

DHA-mediated modulation of selected apoptosis regulatory proteins and pathways

Dysregulation of programmed cell death (apoptosis) is one of the main events in CRC development and progression. The extrinsic apoptotic pathway is activated by specific death ligand - induced receptor oligomerization at the cell surface membrane, causing formation of the death-inducing signalling complex (DISC) and the resulting activation of caspases. The intrinsic pathway involves changes in mitochondrial structure and function, leading to the release of cytochrome c and other proapoptotic factors from mitochondrial intermembrane space that are useful for subsequent caspase activation and apoptosis execution. DHA was identified as a potent apoptosis inducer in colon cancer cells with distinct molecular phenotypes, whereas no significant proapoptotic effects could be seen in normal human colon mucosal epithelial cells NCM460 using physiologically relevant concentrations⁶⁶. This suggests the potential usefulness of DHA as a relatively selective anticancer compound. However, the precise molecular mechanisms of DHA-induced apoptosis remain to be fully clarified.

DHA induced caspase-dependent apoptosis in colon adenocarcinoma HT-29 cells and especially in the more prone adenoma LT97 cells. Caspases-dependent cell death was demonstrated using specific inhibitors. There was also evidence of increased activation of the intrinsic apoptotic pathway demonstrated by the caspase-9 and Bid cleavage⁷⁰. The major involvement of the intrinsic pathway after DHA treatment was further confirmed in other reports where increased expression and activation of Bax and Bak, depolarization of the mitochondrial membrane, and the subsequent release of cytochrome c and Smac/Diablo into the cytosol were reported⁷¹.

Mitochondria are a major subcellular site where n-3 fatty acids are rapidly incorporated. DHA physically interacts with mitochondrial membranes and alters their permeability by opening the permeability transition pores and decreasing the mitochondrial membrane potential (MMP) (ref.⁷²). There is evidence from *in vitro* or *in vivo* studies that DHA is specifically incorporated into phos-

pholipid cardiolipin (CL) (ref.⁴⁵), which is not present in other cell membranes⁷³. DHA caused CL structural changes and increased mitochondrial membrane unsaturation, its susceptibility to oxidation and oxidative stress. This decreases CL binding affinity for cytochrome c and facilitates the release of this and other proapoptotic factors, such as the apoptosis-inducing factor (AIF), Smac/Diablo, Omi/HtrA2, and endonuclease G, from mitochondria to cytosol⁷⁴. Once these factors are released from mitochondria, apoptosis is accelerated⁷⁵. These findings were confirmed *in vitro*²² as well as *in vivo*⁵¹.

As mentioned above, changes in mitochondria can influence the total level/ activity of pro- and antiapoptotic proteins of the Bcl-2 family associated with these organelles. It has been demonstrated that DHA contributes to down-regulation of Bcl-2, a well-known antiapoptotic molecule⁶¹ which, among its other functions, can block lipid peroxidation and thus apoptosis induction⁷⁶.

DHA can also act as an efficient modulator of the level and activity of endogenous caspase inhibitors. DHA and EPA decreased XIAP (an X-linked inhibitor of apoptosis protein) at both protein and mRNA levels which could be of critical importance in their antineoplastic effects on CRC. High XIAP expression correlates with poor clinical outcome, resistance to chemotherapy and radiotherapy in different colon cancer cell lines⁷¹. DHA was shown to down-regulate mRNA as well as the protein level of two other inhibitors of apoptosis, survivin and livin, in cancer cells⁷⁷. Further, the immediate and dramatic down-regulation of FLIP, a potent inhibitor of caspase-8 activation, appears to be a significant event in the induction of apoptosis in colon cancer cells after DHA and EPA supplementation⁷¹.

Impact of DHA on cellular oxidative metabolism

Antiproliferative and apoptotic effects of highly unsaturated DHA were shown to be associated with induction of oxidative stress. DHA can affect the production of hydrogen peroxide, superoxide and lipid peroxidation products, which are involved in regulation of the cell cycle and apoptosis. The balance between oxidative and reducing events plays an important role⁷⁸. Oxidization of DHA through enzymatic processes (see below) or non-enzymatic pathways generates an array of different products (such as isoprostanes and aldehydes) which contribute to DHA anticancer activity. The impact of oxidized products also depends on cell antioxidant ability which is lower in tumour cells⁷⁹.

In vitro studies using human cancer cell lines demonstrated that DHA significantly enhanced lipid peroxidation, which was completely abolished by the antioxidant vitamin E (α -tocopherol) (ref.⁸⁰). Moreover, DHA can inhibit the expression of antioxidant enzymes or deplete cells of antioxidants⁸¹. It appears that susceptibility to the oxidative stress induced by DHA is influenced by complex interactions among multiple antioxidant systems⁸².

The results from our own experiments comparing human colon adenocarcinoma HT-29 and HCT-116 cells with fetal colon FHC cells, confirmed the induction of ROS and lipid peroxidation triggered by a relatively low

dose of DHA (50 μ M). The effect depended on the cell type. Only in the FHC cells with the highest oxidative response was this accompanied by a certain level of apoptosis which was suppressed by the antioxidant Trolox⁸³. Recently, it was reported that the toxicity of high DHA doses (500 μ M) to HT-29 cells is attributable to lipid peroxidation with different roles played by the internal and terminal double bonds of DHA (ref.⁸⁴).

DHA can inhibit the production of nitrogen oxide (NO) by inhibition of inducible nitrogen oxide synthase (iNOS) as shown in CaCo-2 cells. Importantly, the fewer iNOS-positive cells observed in this study are consistent with an increase in number of apoptotic cells and this implies a protective role of NO in this process⁸⁵.

Effects of DHA on eicosanoid metabolism

Cancer development is often associated with activation and changes in the expression of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes controlling the synthesis of eicosanoids derived from AA (ref.⁸⁶). These processes can be modified by various dietary substances including PUFAs (ref.^{87,88}). Most of the beneficial effects of n-3 PUFAs, particularly their antithrombotic, antiinflammatory and also anticarcinogenic effects, are attributed precisely to their inhibition of AA metabolism. Of PUFAs, EPA, competes for the same enzymes to form less bioactive 3-series prostaglandins (PGs) and thromboxanes, and the 5-series leukotrienes (LTs). Recently, using the lipidomics approach, other biologically potent mediators derived from both EPA and DHA called resolvins have been found. DHA metabolites comprise several resolvins of the D series (RvD1-4) and protectins. These endogenous agonists possess pre-resolving, antiinflammatory, antifibrotic, and host-directed antimicrobial actions that are cell-type specific⁸⁹.

It has also been suggested that DHA may have proapoptotic effects in colon cancer cell lines by inhibiting the expression and activity of the enzyme COX-2 (ref.⁹⁰), which is often overexpressed in colon tumours and is able to confer resistance to apoptosis. Moreover, DHA may suppress tumour cell growth directly by inhibition of the COX-2 product PGE₂ which stimulates cell proliferation⁹¹. Although these results are limited to an *in vitro* setup, they increase evidence for the argument that the ratio of n-6/n-3 PUFAs (and in particular the ratio of AA to DHA) is a critical determinant of proliferation and tumour growth in the colon, and that DHA supplementation can suppress tumour cell growth, even in the presence of high AA and PGE₂ levels⁹¹. However, it is possible that DHA may also act via mechanisms independent of COX-2 inhibition⁹², because it suppresses growth of a colon tumour cell line, which does not express COX at the protein level. Moreover, the growth of these cells in culture and in nude mice was not affected by overexpression of COX-1 or COX-2 (ref.⁹³).

Effects of DHA on cell cycle regulatory proteins

A number of studies have described modulation of cell cycle control genes after DHA treatment. Several key transcripts involved in the regulation of both the G1 and G2

phases of the cell cycle were affected by DHA treatment in the SW620 cell line derived from lymph node metastasis of colon cancer. Generally, molecules involved in cell cycle progression, such as Cdc25c, Cdc25b, Cdc20, CDK1, CDK2, and cyclin D, A, and B, were down-regulated, whereas molecules involved in cell cycle arrest such as p21 and stratifin were up-regulated⁷⁷. SW620 cells and cell line SW480, derived from primary tumour of the same patient, accumulated DHA differently in TGs and/or cholesterol ester-enriched lipid droplets. DHA caused cell cycle arrest and down-regulated the nuclear form of sterol regulatory element-binding proteins (SREBP1 and 2) in these cells, indicating a possible relationship between disturbances in lipid homeostasis and cell cycle arrest^{80,48}.

While a large number of mechanisms have been linked to DHA antiproliferative effects in cancer, several reports have focused on whether p53 protein plays a role in DHA-induced growth inhibition. DHA inhibited the growth of p53-wildtype colon cell lines (LS-174 and Colo 320 HSR) as well as of those with different p53 mutations (HT-29 and Colo 205); thus its action does not seem to be dependent on p53 status⁹⁴. However, another study showed that DHA *in vitro* effects may be p53-dependent in cell lines expressing wildtype p53. Moreover, the ability of DHA to inhibit colony formation in soft agar may be unrelated to p53, raising the question how relevant these findings are to *in vivo* models⁹⁵.

Regulation of specific transcription factors and DHA effects on gene expression

PUFAs and their various metabolites have been shown to interact with specific transcription mediators including nuclear peroxisome proliferator-activated receptors (PPARs), the retinoid X receptor (RXR) α , the hepatocyte nuclear factor (HNF)-4 α , the liver X receptor (LXR), SREBPs, and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) (ref.⁹⁶). Three members of the PPAR family (PPAR α , PPAR β , and PPAR γ) have been shown to be major targets for fatty acids⁹⁷ and were found to be involved in the regulation of lipid metabolism and storage in adipose tissue, β -oxidation of fatty acids in peroxisomes and mitochondria and energy balance⁹⁸. PPAR β and impaired expression and function of PPAR γ are associated with inflammatory bowel disease (IBD) and colon cancer⁹⁹. DHA can be transported to the nucleus where it interacts mainly with PPAR γ . It regulates fatty acid homeostasis in part through the regulation of expression of membrane-bound fatty acid transporting proteins and LD accumulation. Activation of PPAR γ as well as heterodimers formed with RXR are reported to play an important role in the antitumour effects of n-3 PUFAs (ref.⁹⁹).

Regulation of the NF- κ B activity is another target of the major n-3 fatty acids in fish oil¹⁰⁰. Activation of NF- κ B and the PPAR-Bcl-2 feedback loop may control the life-death continuum in colon cells and can be further associated with the expression of COX-2 (ref.¹⁰¹). Suppression of the activation of NF- κ B by DHA not only reduced the production of pro-proliferative eicosanoids produced by COX-2 it also decreased the production of other NF- κ B-

induced cytokines that promote cancer cell growth¹⁰². DHA - decreased NFκB activity also sensitized tumour cells to gamma irradiation and induction of apoptosis⁹⁹.

Some colon adenocarcinomas have defective expression of the adenomatous polyposis coli (APC) gene, which is a component of the Wnt signalling pathway. This and other developmental pathways play an important role in genetic and sporadic epithelial cancers¹⁰³. *In vivo* studies demonstrated that fish oil – derived concentrate suppressed formation of intestinal tumours in mice with a defective APC gene. The downstream APC signalling oncogene is cMyc, an important regulator of cell proliferation. Lack of cMyc expression was associated with a reduced number of intestinal adenomas¹⁰⁴. On the other hand, patients with amplified cMyc gene and wild type p53 have a greater response to anticancer treatment¹⁰⁵. DHA was shown to increase the level of cMyc, which was related to apoptosis induction in colon cancer cells⁹⁴.

Important information was obtained from DNA microarrays which indicated reprogramming of a large number of genes and transcription factors after n-3 PUFA treatment, e.g. altered expression of RNA II polymerases, elevated expression of proapoptotic caspases, and activation of cyclin-dependent kinase inhibitors (p21, p27, p57, p19) (ref.¹⁰⁶). Inactivation of the PG family of genes, LOXs, and altered expression of PPARs were detected¹⁰⁷. Similarly, cDNA arrays after EPA and DHA treatment using a colon adenoma cell line revealed changes in expression of several gene types, such as those of detoxification, cell cycle control, signalling pathways, apoptosis and inflammation, confirming the chemoprotective effects of these PUFAs (ref.³²). Both EPA and DHA decreased the growth of colon tumours in nude mice by reducing vascular endothelial growth factor (VEGF) and COX-2 expression through inhibition of ERK1/2 phosphorylation and hypoxia-induced factor (HIF)-1 α protein expression¹⁰⁸. Taken together, these results confirm that dietary fat composition alters the molecular portrait of gene expression profiles in colonic epithelium at both initiation and promotion stages of colon carcinogenesis and indicate that these chemopreventive effects are due to direct action of n-3 PUFAs.

Antimetastatic and antiangiogenic activity of DHA

Tumour cells pretreated with fatty acids, in particular with DHA, and then introduced into animals showed a low potential for lung colony formation. Increased apoptosis induction of metastatic tumours after DHA treatment was also observed⁴⁹. This activity may be related to changes in the fatty acid composition of tumour cells, which impaired the tumour cell membrane and reduced the ability to metastasize¹⁰⁹. Moreover, impaired attachment to endothelial cells and the indicated decrease in metalloproteinase-9 activity in tumour cells may play a role in these effects¹¹⁰.

Some results from *in vivo* experiments confirm the importance of the antiangiogenic activity of DHA (ref.⁵²). EPA and DHA have potent antiangiogenic effects by inhibiting production of many key angiogenic mediators, namely VEGF, the platelet-derived growth factor, COX-2,

PGE₂, nitric oxide, NFκB, matrix metalloproteinases, and beta-catenin¹¹¹.

Data from *in vitro* and *in vivo* studies have revealed a potential antiangiogenic role of n-3 PUFA in intestinal endothelial cells. DHA decreased vascular cell adhesion protein 1 (VCAM-1), Toll-like receptor 4, COX-2 and VEGFR2 expression, production of interleukins (IL-6, IL-8), GM-CSF, and reduced production of PGE₂ and LTB4 in IL-1 β -induced human intestinal microvascular endothelial cells (HIMEC). Similarly, dietary intervention with fish oil rich in EPA and DHA significantly decreased colon production of PGE₂, endothelial VCAM-1, and VEGFR2 in rats with induced colitis. This protective effect of n-3 PUFAs may partly account for the observed benefit of dietary intake of n-3 PUFAs in IBD (ref.¹¹²).

Although DHA shows antiproliferative and apoptotic ability in many experimental systems, its positive effects would be more expected in prevention than in direct therapy. It appears to be effective especially as an anti-inflammatory agent and in cases of precancerous lesions. Apropos treatment, the supporting effects of this dietary fatty acid in appropriate combination with other factors and/or classical chemotherapy are overviewed in the following section.

DHA SENSITIZES COLON CANCER CELLS TO VARIOUS ANTICANCER AGENTS

Until now, different cancer treatments have used drugs with serious side effects and are often toxic to healthy tissues. For this reason, much effort is directed to find better-tolerated treatments. From the clinical viewpoint, DHA looks promising in cancer prevention as well as in cancer treatment. Both experimental and several clinical studies have shown that DHA or agents containing DHA can improve not only parameters accompanying inflammation and neoplastic diseases, but can also enhance tumour responsiveness to anticancer therapy¹¹³. This includes radiation, hyperthermia, chemotherapeutic drugs such as doxorubicin, epirubicin, CPT-11, 5-fluorouracil (5-FU), tamoxifen, and photodynamic therapy^{114,115}. In addition, DHA may also prevent or reduce some of the side effects of the cancer therapies and cachexia¹¹⁶. Thus, it is generally regarded as safe, well-tolerated and without negative impact on patients or normal tissue. It is promising as a non-toxic adjuvant to standard cancer therapies, but further studies are needed for use of DHA in practice¹¹⁷.

Potential of anticancer chemotherapeutic drug effects by DHA

Several excellent reviews have recently been published summarizing the antineoplastic effects of DHA in combination with radiation and chemotherapy in several types of cancer including colon^{118,119}. Increased membrane fluidity, elevated unsaturation index, enhanced transport capabilities or enhanced oxidative stress caused by DHA can improve the uptake, intracellular distribution, and effects of chemotherapeutic drugs. This results in increased sensitivity to chemotherapy, especially in resistant tumour

cells, and causes selective enhancement of cytotoxicity to tumour cells and protection of normal cells¹²⁰.

Paclitaxel, a taxane diterpenoid, is one of the most common anticancer drugs for the treatment of various types of solid tumours. Conjugates of paclitaxel and DHA are stable in plasma and cause regression of tumours with a favourable toxicity profile¹²¹. Promising results were achieved with conjugates of newly developed second-generation taxoids with DHA which were efficient *in vivo* against drug-resistant ovarian and colon xenografts¹²². Generally, chemotherapeutic drugs conjugated to fatty acids could enhance tumour targeting and delivery of prodrugs for intratumoral activation. However, additional clinical experience is needed.

DHA triggers oxidative stress which can increase the sensitivity of cancer cells to drugs producing ROS, such as doxorubicin. This result was obtained in a breast cancer model. There are no comparable data available for colon cancer⁵⁸. Pretreatment with DHA is reported to enhance oxidative stress (namely lipid peroxidation and reactive nitrogen species production) and induction of apoptosis in a human colon cancer HT-29 cell line exposed to hypericin-mediated photodynamic therapy¹²³. DHA also enhanced the apoptotic effect of arsenic trioxide (As_2O_3) in resistant solid tumour cell lines including colon by increasing intracellular ROS and lipid peroxidation products. The effect seems to be tumour-specific because no reduction in cell viability was observed in normal skin fibroblasts¹²⁴.

Some studies have shown that altering tumour fatty acid composition by DHA enrichment changes the drug-resistant phenotype to a drug-sensitive one¹³. Platinum-based drugs are preferentially used for the treatment of different types of cancers (ovarian, head and neck, small cell lung, and colon) (ref.¹²⁵). Acquired resistance to these drugs is a serious clinical problem. Therefore, the option of supporting the activity of cisplatin by a fish oil diet under a conditioned balance of oxidation and antioxidation adjusted by vitamins E and C is of interest¹²⁶. Recently, it was shown that a combination of lipid capsules containing DHA with standard platinum-based chemotherapy increased the response rates and clinical benefits in patients with advanced non-small cell lung cancer¹²⁷.

Fish oil-based lipid emulsion rich in DHA, which is used in humans as a component of parenteral nutrition, evoked accumulation of colon cancer cells in the G2/M cell cycle phase and induced apoptosis. Moreover, combined treatment with 5-FU resulted in a significant enhancement of the cell growth inhibition¹²⁸. Increased efficacy was also found after combined treatment with low doses of DHA and 5-FU which was linked to inhibition of the antiapoptotic proteins Bcl-2 and Bcl-xL (ref.⁹⁴).

The topoisomerase I inhibitor, CPT-11 (irinotecan), is a very effective chemotherapeutic drug, but with severe side effects, especially on the intestine and bone marrow. There are some studies using a DHA-containing product in combination with CPT-11 demonstrating reduction of these side effects *in vivo*¹⁰². In an animal model of the Ward colon tumour, n-3 PUFA enhanced antitumour effects and improved the outcomes of combined CPT-

11/5FU chemotherapy¹²⁹. For severe colon cancer patients with liver metastasis, combined chemotherapy of oxaliplatin, irinotecan, 5-FU, and leucovorin has been developed. However, this also has many side effects. Trials using supplementation of DHA would thus be relevant¹³⁰.

A highly promising approach is the use of low doses of COX-2 inhibitors in combination with DHA. Treatment with subtoxic concentrations of celecoxib and DHA suppressed proliferation in human colon cancer cell lines *in vitro* and induced apoptosis in a dose-dependent manner¹³¹. Celecoxib given *in vivo* together with a high-fat diet containing fish oil caused a significant inhibition of the expression and activity of COX-2 and tumour incidence¹³².

DHA increased the antitumour efficacy of synthetic organoselenium 1,4-phenylene bis(methylene) selenocyanate (p-XSC) in colon cancer cell lines. These effects were mediated by a cascade of events with a primary inhibitory effect on the expression of beta-catenin, followed by a reduced expression of NF- κ B, COX-2, and iNOS. The above cellular effects render the cells susceptible to apoptosis and result in cell growth inhibition⁶⁸.

Effective anticancer therapy appears to be not only a question of toxicity but also of immunogenic response to the particular drug used. For this reason, an effective therapy should harmonize tumour cell elimination with antitumour host immune response. There are studies demonstrating that some anticancer drugs such as anthracyclines have these properties¹³³. A recent study reports that DHA improves the effect of immunogenic response in cancer cell lines as well¹³⁴.

Although the above-described effects of DHA are generally beneficial, it is necessary to consider the potential harmful effects, particularly in high doses. DHA was shown to influence the immune system and inflammatory pathways, and it is the source of a number of oxidative products which may have deleterious effects on non-tumour tissues, too. DHA has been reported to decrease lymphocyte proliferation¹³⁵ and reduce NK cell activity¹³⁶, which is questionable especially for immune therapies, such as those using monoclonal antibodies. In higher concentrations (100 μ M) DHA can inhibit reactive oxygen and nitrogen species formation and reduce expression of iNOS in stimulated macrophages¹³⁷. However, the problem of immunological studies using DHA is that different parameters and experimental systems were used. The question of DHA as a beneficial adjuvant in human tissues is controversial and has been recently reviewed by Serini et al.¹³⁸.

In summary, even if there are studies demonstrating some negative effects of DHA on the human body (especially in very high doses or in processing fish oil), this fatty acid can be considered a relatively non-toxic form of supportive therapy for improving cancer treatment outcomes and slowing down or preventing cancer recurrence. Changes in membrane properties, targeting mitochondria, and modulation of redox balance are presumed to be important mechanisms for increasing the efficacy of many types of therapies with DHA.

Interaction of DHA with butyrate

It has been proposed by ourselves and others, that PUFAs and the short-chain fatty acid butyrate may operate together in the colon stimulating mutual beneficial effects. Interaction of these dietary compounds in the colonic lumen can have substantial impact on the metabolism and kinetics of the colon epithelial cell population and may influence inflammation and neoplastic changes. Butyrate, a four-carbon short-chain fatty acid, is produced during anaerobic fermentation of dietary fibre by endogenous bacteria present in the colon. It is an interesting biologically active agent with a wide variety of effects¹³⁹. It acts as a principal energy source and a survival factor for normal colon cells, whereas it exerts antiproliferative, differentiation- and apoptosis-inducing effects in cancer cells¹⁴⁰. In addition to the regulation of basic cytokinetic processes, butyrate has also been shown to affect cell adhesion, morphology, invasiveness, metastasis, oxidative metabolism, angiogenesis, and the activity of different enzymes and transcription factors. Butyrate functions as a histone deacetylase inhibitor and thus influences gene expression¹⁴¹.

Studies published by a group from Texas University from the early 1990s until now have described the protective effects of fish oil containing DHA, compared to corn oil and its interaction with fibre using rat and mouse model colon carcinogenesis¹⁰. The researchers found that the effects on colon cell proliferation depend on the type of fat (corn oil, fish oil or beef tallow) and fibre (pectin, cellulose) as well as of their combinations and reported several mechanisms of the effects observed. They showed that an important aspect of common DHA and butyrate effects is potentiation of the intrinsic mitochondrial apoptotic pathway¹⁴². Mitochondria also play a very important role in the maintenance of Ca²⁺ homeostasis, which is synergistically imbalanced by DHA and butyrate treatment, causing mitochondrial Ca²⁺ accumulation and peroxidation which triggers apoptosis in a p53-independent manner¹⁴³. Dietary fish oil and pectin protect against radiation-enhanced CRC by up-regulating apoptosis. This was associated with suppression of the antiapoptotic COX and Wnt-beta-catenin pathways, suppression of PPAR δ and PGE₂, and elevation of PGE₃ (ref.¹⁴⁴). Recently, yet other targets of DHA protective effects, intestinal non-coding RNA (microRNA) have been identified. N-3 enriched chemoprotective diet (with fish oil and fibre) modulated miRNA and mRNA expression profiles in the rat colon, suppressing the effects of AOM treatment¹⁴⁵.

For several years, our laboratory has focused on the effects of model PUFAs of n-6 (AA) and n-3 (DHA) types and sodium butyrate (NaBt) on various types of human colon epithelial cell lines *in vitro*. We showed that combined PUFA and NaBt treatment enhanced cell cycle arrest and may shift the balance between differentiation and apoptosis depending on the cell transformation level^{183,146}. Our results thus support the concept of modulation of NaBt effects by PUFAs, especially DHA, in colonic cells. We demonstrated that cytokinetic modulation induced by fatty acids were accompanied by membrane lipid structure changes, LD accumulation, ROS production, decreased

MMP, modulation of caspases activation, PARP cleavage, and changes of Mcl-1 antiapoptotic protein expression. Recently, using the lipidomics approach, we reported significant alterations in cellular lipid and fatty acid composition and metabolism associated with the described effects after treatment with NaBt, DHA, AA or their combination¹⁴⁷.

Interaction of DHA with the effects of selected TNF family cytokines

Mechanisms influencing the effects of specific endogenous molecules regulating cell growth and death (growth factors, cytokines) are assumed to play a role in DHA anticancer effects. It has been shown that n-3 PUFAs can affect the cellular response to various cytokines, including the tumour necrosis factor (TNF) family members. Within this family, TNF- α , Fas (CD95), and the TNF-related apoptosis inducing ligand (TRAIL) are crucial in the regulation of inflammation and/or cell death. DHA has been reported to influence both TNF- α synthesis as well as its intracellular proinflammatory signalling pathways¹⁴⁸. Here we preferentially refer to the role of DHA as an efficient modulator of cell death induced by selected TNF family cytokines which may significantly affect the progression and/or treatment of various disorders including cancer.

The TNF family members are potent inducers of an extrinsic apoptotic pathway that is triggered via specific surface death receptors, associated with activation of key initiator caspase-8, followed by cleavage of the effector caspases and their substrates¹⁴⁹. Our results demonstrated a different response of human colon cell lines with various carcinogenic potential to TNF-family apoptotic inducers⁷⁸. We also reported that DHA can alter the response of human colon cancer cells so that they become more responsive to apoptosis induced by CH-11 (anti-Fas antibody) (ref.¹⁴⁶). The sensitizing effect of DHA was associated with stimulation of oxidative metabolism and proapoptotic events at the level of mitochondria. On the other hand, a protective role of DHA against various cell death types such as apoptosis and necrosis triggered by TNF- α has been reported in other cell types such as human monocytes or fibrosarcoma, respectively¹⁵⁰. Based on these results, the modulatory effect of DHA on death ligand-induced apoptosis appears to depend significantly on the cell type (tissue of origin, normal/cancer) and its genetic background.

Interestingly, we also showed that DHA can act as an effective stimulator of apoptosis induced by TRAIL in human colon cancer cells¹⁵¹. As TRAIL is a very most promising cytokine of the TNF family for its anticancer application and selectivity¹⁵², the ability of DHA to enhance its antitumour cytotoxic effects would be of crucial importance in both cancer prevention and therapy. In addition, co-application of DHA with TRAIL could increase the success of this kind of therapy in TRAIL-resistant tumour types as well. DHA-mediated enhancement of TRAIL-induced apoptosis was accompanied by a more effective triggering of the mitochondrial apoptotic pathway, reinforcement of caspase activation, cleavage

of caspase substrates, and apoptosis execution¹⁵¹. Other detailed molecular mechanisms of the sensitizing action of DHA in colon cancer cells treated with TRAIL are currently under intense investigation in our laboratory.

While DHA itself (used in a higher concentration range) seems to preferentially engage the intrinsic apoptotic pathway, a large number of studies confirm that, when applied (in lower doses) in combination with the specific death ligands, it can significantly interfere with the extrinsic signalling pathways at various intracellular steps. In addition, the simultaneous triggering of the two apoptotic routes (extrinsic and intrinsic) following combined application of these compounds, might improve future treatment aimed at selective elimination of various undesirable cancer cell types.

PRACTICAL APPLICATIONS

In summary, from the existing knowledge it is clear that lipids or essential PUFAs represent more than just an energy source. Together with cytokines and hormones they function as mediators and modulators of the cellular signalling network and play an important role in physiological and pathophysiological processes. For this reason, it is necessary to consider lipids as pharmaceutical agents¹⁵³ which can have beneficial (or detrimental) impact on the body and can influence the effects of other drugs as described in section III.

A wide field of interest is the composition and optimization of clinical nutrition especially for oncological patients. There are many reports showing the advantage of n-3 PUFAs as a component of various types of enteral and parenteral lipid emulsions. This is why lipids in clinical nutrition can influence inflammation, susceptibility to infection, immune cell function, and thus affect immunological response, cachexia, and exert beneficial effects on the whole organism, which can then fight better against inflammation and cancer¹⁵⁴. Lipid emulsions containing n-3 PUFAs like Omegaven, SMOFlipid or artificial structural triglycerides are well tolerated, modulate the fatty acid and eicosanoid pattern and oxidative metabolism in the body, suggesting anti-inflammatory, immunosupportive and other favourable effects¹⁵⁵. Parenterally applied Omegaven improved intestinal recovery after ischemia-reperfusion injury in rat model¹⁵⁶. In addition to fish oil, algal oil seems to be a better source of n-3 PUFAs especially in high-dose therapy, owing to gastrointestinal problems with of fish oil¹⁵⁷.

Nutritional formulas for improving immunity (immunonutrition) of patients with gastrointestinal cancer contain, apart from n-3 PUFAs, L-glutamine, L-arginine, and RNA. Preoperative, perioperative and postoperative administration of PUFA-enriched nutrition (oral, enteral or parenteral) could have an impact on the postoperative inflammatory response after major abdominal surgery in cancer patients or laparoscopic colorectal surgery¹⁵⁸. Generally, in clinical trials, trends toward decreased infection, hospital stay and treatment costs have been found after preoperative immunonutrition. However, the route,

time and duration of administration have to be considered for individual diagnosis¹⁵⁹.

We investigated the *in vitro* impact of various dilutions of five clinically used parenteral lipid emulsions containing long-chain triglycerides (LCT) (Elolipid, Intralipid Nutralipid P) or mixed LCT and medium-chain triglycerides (MCT) (Lipofundin LCT/MCT and Nutralipid MCT) from soybean and coconut oils. Our results pointed to a different response of colon cell lines of distinct origin. Treatment with emulsions increased the percentage of floating cells and subG0/G1 population (indicating induction of cell death) in human colon fetal FHC but not in adenocarcinoma HT-29 cells. This was due especially to the higher oxidative response (increased ROS production and lipid peroxidation) of FHC compared to HT-29 cells¹⁶⁰.

Cancer anorexia and cachexia are major factors that exacerbate the already compromised immune system of cancer patients. Malnutrition of cancer patients is associated with poor prognosis. Supplementation with enteral nutrition enriched with n-3 PUFAs has demonstrated an increase in appetite, energy intake, nutritional status, and improved tolerance to cancer treatments¹⁶¹. Experimental and randomized clinical studies confirmed rapid incorporation of EPA and DHA into the membranes of not only various normal cell types (erythrocytes, liver, gastrointestinal tract, lungs or brain) but also in the tumour tissue of gastrointestinal cancer patients with a consequent decrease in AA content¹⁶². N-3 PUFAs compete with AA and decrease the synthesis of highly active mediators (such as PGE₂ or LT₄) in favour of less active and anti-inflammatory (LT₅, resolvins). They also decreased the production of proinflammatory and procachectic cytokines such as TNF- α , IL-1, and IL6 and results in weight gain compared with placebo¹⁶³.

Of major importance is the ability of n-3 PUFAs to improve conditions in IBD (ulcerative colitis and Crohn disease) decreasing disease-related inflammatory markers such as LTB₄, interferon γ , and IL-2. Supplementation with DHA and EPA allows us to use lower doses of corticoids in therapy¹⁶⁴.

CONCLUSIONS

It can be summarized that dietary lipids containing n-3 PUFAs, particularly DHA, can significantly affect maintenance of cell population homeostasis in the colon, and thus they are involved in the processes leading to pathological states including colon inflammation and cancer. They may function mainly by non-genotoxic mechanisms during cancer promotion and progression. The effects of DHA on cellular and molecular levels are very complex and involve changes of cellular membrane properties and functions, redox balance, production of specific metabolites, activation or suppression of specific signalling pathways, transcription factors, and gene expression, which finally modulate cell behaviour and response to other dietary compounds and endogenous regulators of inflammation, growth and death.

From the data described in this review it appears that use of DHA (alone, together with EPA or in the form of fish or algal oil) might be a relatively nontoxic form of supportive therapy for improving cancer treatment outcomes and slowing down or preventing the recurrence of colon cancer. It is especially suitable for combined therapy enhancing tumour responsiveness. However, most observations of PUFA actions originated from *in vitro* or *in vivo* models using cultured cells or rodents. Thus, it is difficult to establish their direct clinical effects. The targeted use of a specific type of PUFA, such as DHA, may have a number of additional benefits both in physiological and pathophysiological conditions. However, further studies of nutritional intervention and clinical trials are needed owing to conflicting results. Above all, these lipids need to be used with caution, based on solid scientific evidence of their mechanisms of action at all levels.

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CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: None declared.

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