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Review

Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies

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Abstract

Linoleic and alpha-linolenic acids, obtained from plant material in the diet are the precursors in tissues of two families with opposing effects which are referred to as “essential fatty acids” (EFA): arachidonic acid (AA) and pentaene (eicosapentaenoic acid: EPA) and hexaene (docosahexaenoic acid: DHA) acids. The role of EFA is crucial, without a source of AA or compounds which can be converted into AA, synthesis of prostaglandins (PGs) by a cyclooxygenase (COX) enzyme would be compromised, and this would seriously affect many normal metabolic processes. COX, also known as prostaglandin endoperoxide synthase (Pghs) or as prostaglandin G/H synthase, is a key membrane bound enzyme responsible for the oxidation of AA to PGs. Two COX isoforms have been identified, COX-1 and COX-2 that form PGH_2, a common precursor for the biosynthesis of thromboxane A_2 (TxA_2), prostacyclin (PGI_2) and PGs (PGD_2, PGE_2, $PGF_{2\alpha}$. COX-1 enzyme is expressed constitutively in most cells and tissues. Its expression remains constant under either physiological or pathological conditions controlling synthesis of those PGs primarily involved in the regulation of homeostatic functions. In contrast, COX-2 is an intermediate response gene that encodes a 71-kDa protein. COX-2 is normally absent from most cells but highly inducible in certain cells in response to inflammatory stimuli resulting in enhanced PG release. PGs formed by COX-2 primarily mediate pain and inflammation but have multiple effects that can favour tumorigenesis. They are more abundant in cancers than in normal tissues from which the cancers arise. COX-2 is a participant in the pathway of colon carcinogenesis, especially when mutation of the *APC* (Adenomatous Polyposis Coli) tumour suppressor gene is the initiating event. In addition, COX-2 up-regulation and elevated PGE_2 levels are involved in breast carcinogenesis. It seems that there is a correlation between COX-2 level of expression and the size of the tumours and their propensity to invade underlying tissue. Inhibition by non-steroidal anti-inflammatory drugs (NSAIDs) of COX enzymes which significantly suppress PGE_2 levels, reduced breast cancer incidence and protected against colorectal cancer. Therefore it is suggested that consumption of a diet enriched in n-3 PUFA (specifically EPA and DHA) and inhibition of COX-2 by NSAIDs may confer cardioprotective effects and provide a significant mechanism for the prevention and treatment of human cancers.

Keywords: Omega-3 PUFA; Prostaglandins; Cyclooxygenases; Cardiovascular; Cancer

1. Introduction

Animal fats contain a high proportion of glycerides of saturated fatty acids and tend to be solids whilst those from plants and fish contain predominantly unsaturated fatty acid esters and tend to be liquids (Table 1).

Polyunsaturated fatty acids (PUFA) can arise by more than one biosynthetic route but in most organisms they arise by desaturation of the corresponding alkanoic acid. Most eukaryotic organisms possess a Δ^9-desaturase that introduces a *cis* double bond into a saturated fatty acid.

In animals, linoleic acid (LA) must be obtained from plant material in the diet and it is desaturated towards the carboxyl group to yield γ-linolenate. Two families with opposing effects which are referred to as “essential fatty acids” (EFA), the (n-6) (arachidonic acid: AA) and the (n-3)

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Table 1
The chemical structure of major saturated and unsaturated fatty acids

Unsaturated Fatty acids
18:1 (11c) Oleic $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
18:2 (9c, 12c) Linoleic $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
18:3 (9c, 12c, 15c) α-Linolenic $CH_3(CH_2)CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
18:3 (6c, 9c, 12c) γ-Linolenic $CH_3(CH_2)4CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$
$CH_3(CH_{2)4}CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
20:4 (5c, 8c, 11c, 14c) Arachidonic
$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$
20:5 (5c, 8c, 11c, 14c, 17c) Eicosapentaenoic (EPA)
$CH_3(CH_2)CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_2COOH$
22:6 (4c, 7c, 10c, 13c, 16c, 19c) Docosahexaenoic (DHA)
Saturated fatty acids
14:0 Myristic $CH_3(CH_2)_{12}COOH$
16:0 Palmitic $CH_3(CH_2)_{14}COOH$
18:0 Stearic $CH_3(CH_2)_{16}COOH$

(eicosapentaenoic acid: EPA) are the precursors of prostaglandins (PGs), thromboxanes and leukotrienes (LT). LA, fed to EFA-deficient rats, is a precursor of AA in tissues and α-linolenic acid also obtained from the diet is a precursor of EPA and docosahexaenoic acid (DHA) [1] (Fig. 1). Since these fatty acids all have double bonds three carbons from the methyl end of the chain, they are grouped under the term of ω-3 fatty acids.

The role of EFA such as linoleic, and γ-inolenic acids obtained from plant ingredients in the diet is crucial. Without a source of AA or compounds which can be converted into AA, synthesis of PGs would be compromised, and this would seriously affect many normal metabolic processes.

PGs occur at very low concentrations in nearly all mammalian tissues. They were first isolated from human and animal semen and initially assumed to be secreted by the prostate gland. They are synthesized from three EFA: γ-linolenic, arachidonic, and EPA (Fig. 1). Some structures elaborated from AA by a cyclooxygenase (COX) enzyme are converted into PGG_2. The acyclic peroxide group in PGG_2 can be cleaved by a peroxidase to yield PGH_2 which occupies a central role and can be modified in several different ways: abstraction of hydrogen atoms which gives rise to PGF_2 whilst capture and loss of hydrogen atoms would provide either PGE_2 or PGD_2 (Fig. 2). PGs have been found to exert a wide variety of pharmacological effects on humans and animals such as contraction and relaxation of smooth muscle of the uterus. PGE_2 is used in obstetrics to induce abortions during the early to middle stages of pregnancy, or to induce labour at term. PGE_1 also has vasodilatory properties and is used for maintaining new born infants with congenital heart defects or facilitating blood oxygenation prior to corrective surgery. It is also of value in male impotence, being used to achieve erection of the penis. PGI_2 reduces blood pressure and inhibits platelet aggregation by reducing calcium concentrations. It is employed to inhibit blood clotting during renal dialysis and inhibit gastric acid secretion. Recently, it has been shown that PGE2 transactivates Epidermal Growth Factor (EGFR) [2].

PGs are rapidly degraded by processes which include oxidation of the 15-hydroxyl to a ketone, reduction of the 13,14-double bond, and oxidative degradation of both side chains. It has been found that some established non-

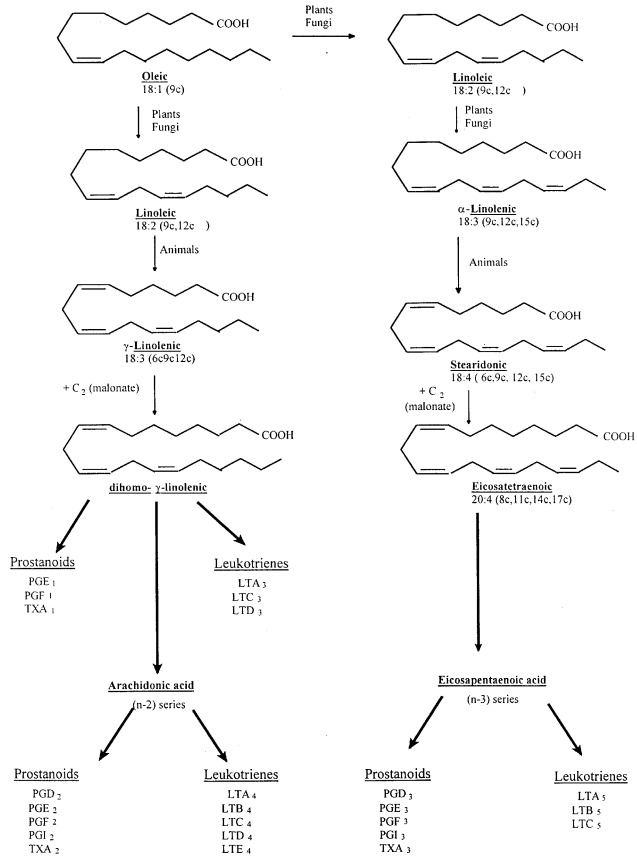

Fig. 1. Thromboxane (see Fig. 3), leukotriene (see Fig. 4) and PG structures representative of the 1-, 2-, and 3-series elaborated from dihomo-gamma-linolenic acid, AA, and EPA, respectively.

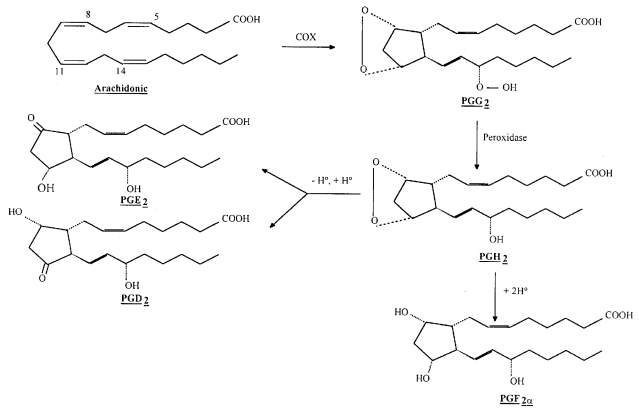

Fig. 2. PG structures representative of the 2-series elaborated from AA. In the first reaction, AA is converted to PGG_2 by COX enzyme which incorporates two molecules of oxygen. The acyclic peroxide group in PGG_2 is then cleaved by a peroxidase to yield PG H_2 (PGH_2) that can be modified in several ways: abstraction of hydrogen atoms gives rise to $PGF_{2\alpha}$ while capture and loss of hydrogen atoms would provide either PGE_2 or PGD_2.

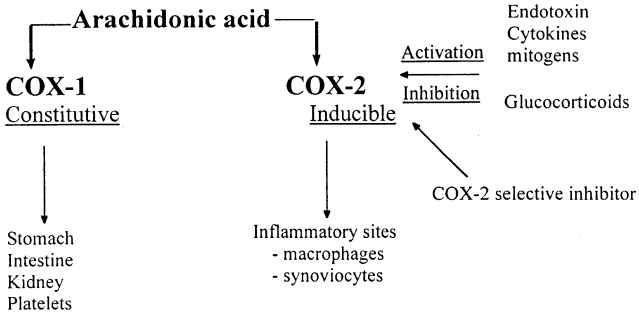

Fig. 3. COXs pathways.

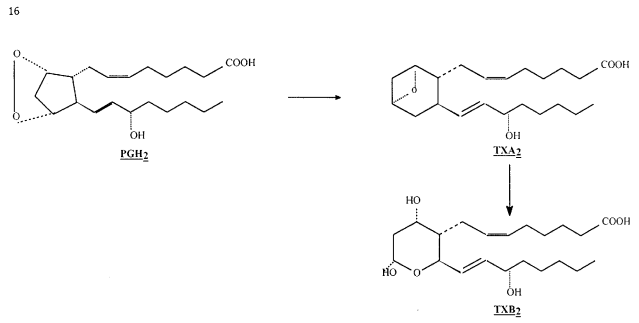

Fig. 4. Formation of thromboxanes. The peroxide and cyclopentane ring functions of PGH_2 are cleaved and restructured to form TXA_2 which is highly unstable. In an aqueous environment, it reacts to yield the hemiacetal thromboxane B_2 (TXB_2).

steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen inhibit early steps in the prostaglandin biosynthetic pathway that transform the unsaturated fatty acids into cyclic peroxides. Thus, aspirin is known to irreversibly inactivate the COX but not the peroxidase activity. Ibuprofen and indomethacin compete with AA at the active site and are reversible inhibitors of COX.

COX, also known as prostaglandin endoperoxide synthase (Pghs) or as prostaglandin G/H synthase, is a key membrane bound enzyme responsible for the oxidation of AA to prostaglandins. Two COX isoforms have been identified, COX-1 and COX-2 that form PGH_2, a common precursor that catalyses the first step in the conversion of AA to prostanoids, including the biosynthesis of thromboxane A_2 (TxA_2), prostacyclin (PGI_2) and PGs (PGD_2, PGE_2, $PGF_{2\alpha}$) (Fig. 2). In many situations, the COX-1 enzyme is expressed constitutively in most tissues and cells to control synthesis of those PGs primarily involved in the regulation of homeostatic functions throughout the body (important for gastrointestinal integrity and vascular homeostasis) and remains constant under either physiological or pathological conditions. In contrast, COX-2 is an intermediate response gene that encodes a 71-kDa protein normally absent from most cells but highly inducible in certain cells in response to inflammatory stimuli resulting in enhanced PG release (Fig. 3). PGs formed by COX-2 primarily mediate pain and inflammation. COX-2 was first described as being induced by a viral oncogene, *v-src* [4] or by a tumour promoter [5] and was shown to be present in epithelial cancers, including gastric [6], oesophageal [7,8], lung [9], colorectal [10] and breast cancers [13], whereas it was absent from adjacent histologically normal intestinal tissue. In contrast, COX-1 was present in both normal and neoplastic tissue equally [10]. COX-2 is also induced by a variety of factors including tumour promoters, cytokines, growth factors and hypoxia [11,12] and is overexpressed in rheumatoid arthritis, colorectal [10] and breast cancers [13]. Its utilization of AA also perturbs the level of intracellular free AA and subsequently affects cellular functions [14]. Oxidation of arachidonate by COX generates other oxidative species and thus raises the overall oxidative state of the cell [15-18]. Based on structural differences in the active sites of COX-1 and COX-2 (which differ minimally, valine/isoleucine substitutions, at only two positions), a new class of drug has been developed that specifically inhibits COX-2 but not COX-1 activity. COX-2 inhibition provides the therapeutic (anti-inflammatory) activity of NSAIDs, whereas inhibition of constitutive COX-1 is responsible for their gastric and renal side effects as well as for their anti-thrombic activity [19-22]. The anti-inflammatory activity of corticosteroids correlates with preventing the release of AA from storage

phospholipids, and glucocorticoids inhibit the expression of COX-2.

A decrease in apoptosis accompanied by increased levels of the anti-apoptotic protein Bcl-2 and reduced levels of proapoptotic proteins (Bax and Bcl-xL) and increased output of PGE_2 was shown in rat intestinal cultured cells transfected with COX-2 gene [23]. These data and a direct effect of PGE_2 on Bcl-2 and apoptosis in cell lines derived from human colon cancer samples suggest a possible molecular mechanism for the proneoplastic effect of COX-2 action [23,24].

Thromboxane synthesis occurs through the PG pathways (Fig. 4). They were originally isolated from blood platelets where TXA_2 causes aggregation. It has the opposite effect to PGI_2 and both compounds are produced from the same precursor, PGH_2, which is converted in platelets to TXA_2 and in the blood vessel walls to PGI_2. Thus, the development of thrombosis reflects an imbalance in these two activities. TXA_3 and TXB_3 are derived from EPA and are structurally analogous to PGs in the 3-series.

LT, also synthesized from AA, are converted into hydroperoxides, the point of oxygenation being C-5 rather C-11 as in the PG pathway (Fig. 5). First isolated from leukocytes, LT are involved in allergic responses and in inflammatory processes. Compounds such as histamine or slow reacting substance of anaphylaxis (SRSA) which are a mixture of LTC_4, LTD_4, and LTE_4 are mediators of allergic reactions (hay fever and asthma). The leukotriene function is eliminated by the degradation of the peptide side chain. LTB_4 appears to facilitate the migration of leukocytes in inflammation and is implicated in psoriasis, inflammation of the bowel and arthritis. Drugs which are receptor antagonists of LTD_4 have been introduced for the prophylaxis of asthma and those inhibiting the formation of LTC_4 and LTB_4 are in clinical trials.

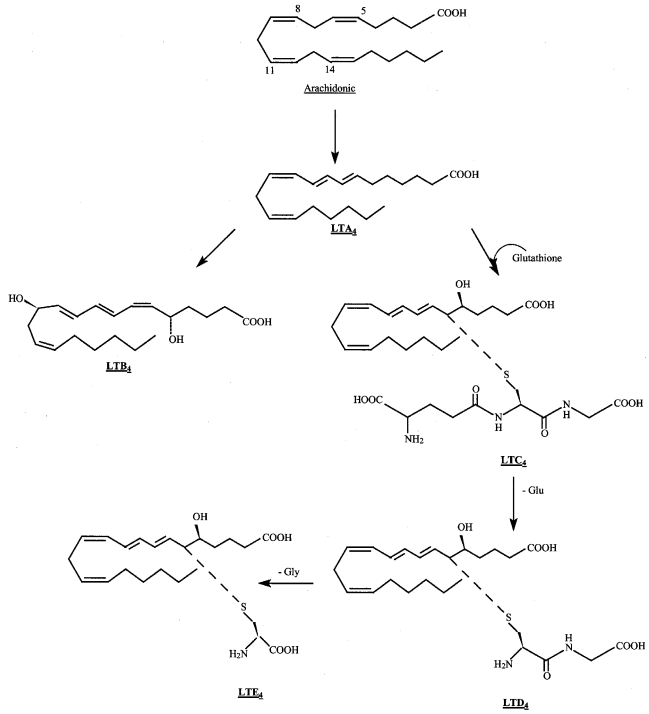

Fig. 5. LT elaborated from AA. LTA_4 is elaborated from AA acid which is converted into a hydroperoxide. The point of oxygenation being C5 rather than C11 as in the PG pathway. LTA_4 is unstable and may hydrolyse giving LTB_4 or may be attacked directly by a nucleophile such as the sulphur atom of glutathione (GSH) to form the LTC_4. Partial hydrolysis in GSH leads to LTD_4 and LTE_4.

2. Human pathologies: protective effects of ω-3 PUFA, and eicosanoids

ω-3 Fatty acids are required for the normal composition of sperm, retina and brain lipids [25] and for the optimal maturation of visual and cortical function in preterm infants [26-32]. It has been shown that DHA deficiency is associated with abnormalities in brain function. n-3 Fatty acids stimulate growth and exert a protective effect on the development of cardiovascular [33], inflammatory symptoms (rheumatoid arthritis, and ulcerative colitis) [34,35], atopic dermatitis and psoriasis [36] and malignant diseases [37]. In the body, AA and EPA are converted to PGs and to LT by COXs and lipoxygenases (5-lipoxygenase: 5-LO), respectively. The AA derived eicosanoids have general proinflammatory effects whereas EPA-derived eicosanoids have anti-inflammatory effects. The anti-inflammatory effects may include decreased production of inflammatory substances like leukotriene B_4 and platelet activating factors (PAF) released by the action of cytokines and a large reduction of cytokine-induced synthesis of PG E_2 and thromboxane B_2 in the colonic mucosa [38,39]. Since AA and EPA have opposing effects in the modulation of inflammation it seems important to determine the (n-6) to (n-3) ratio and not only the absolute level of each family. In inflammatory bowel disease, there are two basic disorders, ulcerative colitis and Crohn's disease. High dietary intake of n-6 PUFA which lowers the intake of n-3 PUFA may contribute to the development of the ulcerative colitis [40], whereas dietary intake of n-3 PUFA, even in low fat diets, ameliorates the intestinal damage of ulcerative colitis [41].

The inflammation experienced in rheumatoid arthritis is due, in part, to hyperproliferation of T cells and the subsequent production of proinflammatory cytokines. Recently, it was shown that consumption of a diet enriched in n-3 PUFA results in down-regulation of IL-2 production in purified T cells stimulated with α-CD3/α-CD28, and in Jurkatt cells stimulated with PMA/ionomycin/α-CD28 [42]. Consumption of EPA reduces the production of interleukin (IL-1) as well as tumour necrosis factor (αTNF) in response to an endotoxin stimulus.

3. Polyunsaturated fatty acids and eicosanoids in cardiovascular pathologies

A growing body of evidence indicates that foods rich in ω-3 PUFAs specifically EPA and DHA confer cardioprotective effects [43,44]. In particular, these substances have been reported to lower blood pressure and prevent the development of hypertension [45-49], one of the most critical factors involved in cardiovascular pathologies such as atherosclerosis or stroke. Although the mechanisms leading to these protective effects remain unclear, it has been suggested that it is through the inhibition of Δ^5 and Δ^6 desaturase activities. These enzymes were reported to convert 20:3(n-6) to 20:4(n-6) and 18:2(n-6) to 18:3(n-6), respectively. The conversion of 18:2(n-6) to 18:3(n-6) is a rate-limiting step in the biosynthesis of 20:3(n-6) from 18:2(n-6). The inhibition of Δ^5 desaturase activity by EPA and DHA will reduce the amount of 20:4(n-6) precursor of TxA_2 (proaggregator) synthesis and increase the amount of 20:3(n-6), precursor of PG E1 (vasodilator) [3]. It is possible to by-pass the enzymatic step by increasing the dietary γ-linolenic acid (18:3 n-6) which is rapidly converted to 20:3(n-6), precursor of PG E1, anti-aggregator and vasodilator.

Other mechanisms may also be involved in PUFA cardiovascular protective effects such as the lowering of platelet aggregation [50]. The (n-3) fatty acids inhibit vasoconstrictor TxA_2 biosynthesis, but blood pressure values are not correlated with plasma TxA_2 concentrations in SHR rats [45]. However, decreased 20:4(n-6) levels in platelet lipids might decrease TxA_2 synthesis and platelet sensitivity, thus resulting in lower cardiovascular risks. The predominant beneficial effects include a reduction in sudden death, decreased arrhythmia, lower plasma triglyceride levels and a reduced blood clotting tendency [51-53]. EPA and DHA provided at high doses (3–4 g/d) can reduce plasma triglyceride levels in patients with hypertriglyceridemia [29]. α-Linolenic acid was also shown to reduce risk of myocardial infarction and fatal ischaemic heart disease in women [54-56]. In contrast, *trans*-unsaturated fatty acids found in prepared foods containing partially hydrogenated vegetable oils can increase LDL cholesterol and reduce HDL cholesterol [57,58].

The most common fatal arrhythmia, ventricular fibrillation (VF), is responsible for death due to coronary artery disease and is caused by disturbances in the electrical stability of the heart [59]. Cardiac arrhythmia occurs during the early and potentially reversible phase of ischaemia and after reperfusion, in most cases without previous symptoms [60]. The risk of ventricular arrhythmia induced by ischaemia was found to be directly proportional to the balance between TXA_2 and PGI_2 (prostacyclin). It has been shown that PGI_2 reduces blood pressure and inhibits platelet aggregation by reducing calcium concentrations. Reduced risk of VF is probably due to a reduced ratio of AA/EPA and a shift of eicosanoid production toward an increase in TXA_3 and PGI_3 at the expense of TXA_2 and PGI_2.The mechanisms by which (n-3) PUFA, EPA and DHA compete with AA include: (n-6) desaturase activity inhibition and consequently inhibition of AA biosynthesis; reduction of plasma and cellular levels of AA by competing with it in membrane phospholipids; EPA competes with AA as the substrate for COX, inhibiting the production of TXA_2 by platelets [61,62]. Moreover, in endothelial cells, PGI_3 is synthesized from EPA which adds on to PGI_2 [63]. Nevertheless, the anti-arrhythmic effect of dietary (n-3) PUFA is independent of atherosclerosis-induced cardiac vulnerability and is exclusively due to its effect on myocardial membranes [64]. Highly polyunsaturated fatty acids are vulnerable to oxidation and this would be expected to increase the risk of cardiovascular disease. Thus, it appears somewhat paradoxical that they are preventive and a definite mechanism by which they prevent these pathologies is not clear at present. The prevention of arrhythmia by (n-3) PUFA is probably due to the interaction of various mechanisms rather than to a single one [65]. It may be mediated via a direct action on the electrophysiological properties of myocytes rather than incorporation of (n-3) PUFA [66]. Moreover, exposure of rat cardiac myocytes to ouabain, a potent arrhythmogenic glycoside, indicated that VF was prevented by the addition of (n-3) PUFA to the culture medium. This effect was attributed to the cytosolic calcium increase effect produced by ouabain [67].

4. Polyunsaturated fatty acids and eicosanoids in cancer

PGs have multiple effects that favour tumorigenesis. They are also more abundant in cancers than in normal tissues from which cancers arise [68]. In rats, LA, a precursor of AA in tissues, increases the size and number of tumours whereas EPA and DHA decrease both [69,70]. It is suggested that the potential of (n-3) fatty acids to prevent recurrence and metastases of mammary cancer when used in adjuvant therapy is associated with a (n-6) to (n-3) ratio < 2:1 [71]. In humans, dietary (n-3) FA treatment offers possibilities in malignant diseases [37]. In contrast, low α-linolenic acid (precursor of EPA and DHA) levels in mammary adipose tissue are associated with an increased risk of breast cancer in women [72]. In patients with prostate cancer, fish intake was inversely related to cancer [73]. In the great majority of colon adenocarcinomas taken from humans, COX-2 levels are 2- to 50-fold higher than levels in adjacent normal intestinal mucosa, while COX-1 levels are unchanged [74,75]. Although the mechanism of action of PUFA is still unclear, the identification of an

enzyme catalysing fatty acid oxidation as a rate limiting step in the progress from normal cell growth through hyperplasia on to neoplasia has opened up a new field of research. COX-2 may be a rate-limiting component at several stages in the development of neoplasia [76,77]. This enzyme is a participant in the pathway of colon carcinogenesis, especially when mutation of the *APC* (Adenomatous Polyposis Coli) tumour suppressor gene is the initiating event [78-80]. Several mutations are known to give rise to defective APC proteins and all who carry the defective gene will develop colorectal cancer [81,82]. In addition, data in human breast cancers as well as animal model studies indicate that COX-2 up-regulation and elevated PGE_2 levels are involved in breast carcinogenesis and that their inhibition by NSAIDs may reduce breast cancer incidence [13,83,84]. It seems that there is a correlation between COX-2 expression and the size of the tumours and their propensity to invade underlying tissues [85,86]. In addition, DHA down-regulates the expression of COX-2 and induces apoptosis [87-91]. The value of COX-2 inhibition has been recognized for the group defined as familial adenomatous polyposis (FAP) [92]. Inhibition of COX activity, decreases eicosanoid production and prevents lung cancer in animal models [93,94]. Using rectal PGE_2 levels as mucosal biomarkers, it was reported that small doses of aspirin (81 mg) inhibit COX enzymes, significantly suppress PGE_2 levels and protect against colorectal cancer [95]. In other types of cancer such as osteoid osteoma, NSAIDs were able to relieve the primary symptom of severe pain at night, and this was thought to be related to a self-limited growth, high levels of expression of PGE_2 and PGI_2 and strong immunohistochemical staining for COX-2 [96]. Thus, consumption of diet enriched in n-3 PUFA, specifically EPA and DHA, and inhibition of COX-2 by NSAIDs may confer cardioprotective effects and provide a significant mechanism for the prevention of human cancers.

Acknowledgements

This review is a part of the dossier that will be published in the next issue of *Biomedicine & Pharmacotherapy* dedicated to the role of polyunsaturated fatty acids and eicosanoids in human health and pathologies (Biomed Pharmacother 2002;56(8)).

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