EUROPEAN JOURNAL OF CANCER 45 (2009) 2077–2086

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Review

The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential

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ARTICLE INFO

Article history:
Received 31 January 2009
Received in revised form 10 April 2009
Accepted 24 April 2009
Available online 1 June 2009

Keywords:
Omega-3 fatty acids
Tumour angiogenesis
Angiogenesis inhibitor

ABSTRACT

Omega-3 fatty acid (omega-3 FA) consumption has long been associated with a lower incidence of colon, breast and prostate cancers in many human populations. Human trials have demonstrated omega-3 FA to have profound anti-inflammatory effects in those with cancer. *In vitro* and small animal studies have yielded a strong body of evidence establishing omega-3 FA as having anti-inflammatory, anti-apoptotic, anti-proliferative and anti-angiogenic effects. This review explores the evidence and the mechanisms by which omega-3 FA may act as angiogenesis inhibitors and identifies opportunities for original research trialling omega-3 FAs as anti-cancer agents in humans. The conclusions drawn from this review suggest that omega-3 FAs in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found principally in oily fish have potent anti-angiogenic effects inhibiting production of many important angiogenic mediators namely; Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), Platelet-Derived Endothelial Cell Growth Factor (PDECGF), cyclo-oxygenase 2 (COX-2), prostaglandin-E2 (PGE2), nitric oxide, Nuclear Factor Kappa Beta (NFKB), matrix metalloproteinases and beta-catenin.

1. Introduction

1.1. *Tumour angiogenesis*

Angiogenesis is the formation of new blood vessels. This process can be physiological and examples include the development of blood vessels *in utero*, or pathological. Pathological angiogenesis includes diabetic retinopathy, and the development of tumours both benign and malignant.

In 1971 Folkman hypothesised that tumour growth is dependent on angiogenesis[1] and subsequently experimental work demonstrated that for a tumour to grow beyond a size of 1–2 mm^3 a substantial new blood supply must develop to support the increasing metabolic requirements.[2–4]

The mechanisms of angiogenesis have been under investigation since 1931 when Clark and Clark observed real-time capillary growth, and are still not fully understood.[5] However, it is known that inflammation, hypoxia and mechanical forces such as sheer stress, stretching and exercise may activate endothelial cells or cause release of growth factors or cytokines which become involved in a process known as abluminal sprouting – the conventional mechanism in which a new blood supply grows from an existing vessel. Fig. 1 demonstrates the phases of sprouting angiogenesis.

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doi:10.1016/j.ejca.2009.04.026

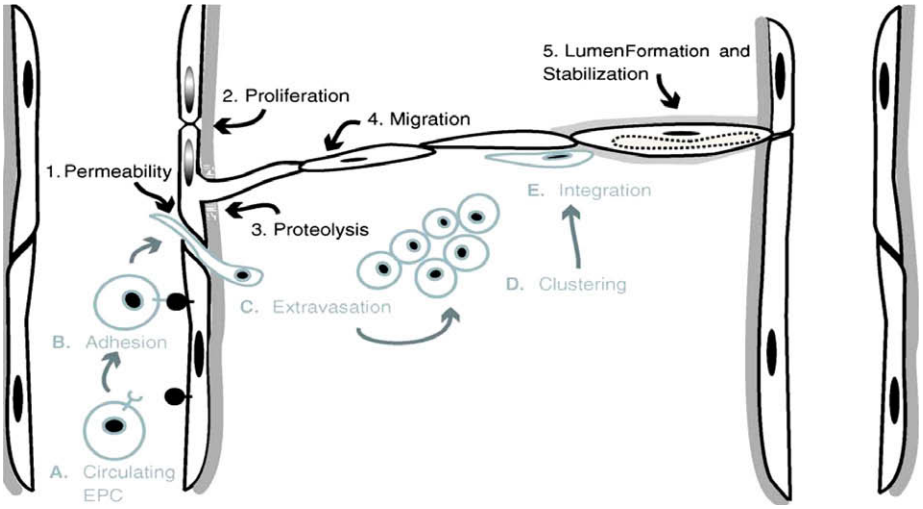

Fig. 1 – Phases of sprouting angiogenesis.[64] The process of abluminal sprouting is initiated by activation of endothelial cells by growth factors, mechanical or inflammatory stimuli. Permeability (1) across the endothelial cell layer increases, and this is followed by proliferation (2). Proteolysis (3) of basement membrane components (controlled mainly by MMPs) enables the sprouting of the endothelial cell into the interstitial space. Continued coordination of cell adhesion and cytoskeletal remodelling components provide directional migration of the sprouting (4) endothelial cells. Proliferation remains greatest at the stalk of the growing sprout. Eventually, the new sprout forms a lumen (5) by the process of intracellular vacuolar fusion or by the stabilisation of several cells around a central lumen. A new lateral branch will be formed when the sprout anastamoses with a pre-existing capillary. Alternatively, circulating EPCs (A) may contribute to the sprout process, adhering (B) to the activated endothelial cell, extravasating (C) through the endothelial cell layer, and clustering (D) within the interstitium. Some of these EPCs will integrate (E) into the sprout and will comprise a portion of the newly formed capillary while others may remain as perivascular cells.

Many molecules such as growth factors and cytokines have both stimulatory and inhibitory roles within sprouting angiogenesis, the most investigated compound being Vascular Endothelial Growth Factor (VEGF) which is known to be a potent stimulator of angiogenesis.[6] Fig. 2 displays the main mediators involved in the angiogenic cascade.

Therapeutic manipulation of tumour angiogenesis is under intense investigation and the search for chemo-preventative agents in the form of angiogenesis inhibitors is an exciting new avenue in cancer prevention.[7]

This quest for angiogenesis inhibitors is not confined to conventional chemo-preventative compounds but extends to substances found in foodstuffs which have long been associated with lower rates of cancer in populations who consume high levels of foods containing these compounds. Examples include; zinc, polyphenols (EGCG) found in green tea and Omega-3 fatty acid (omega-3 FA) principally from oily fish.[8]

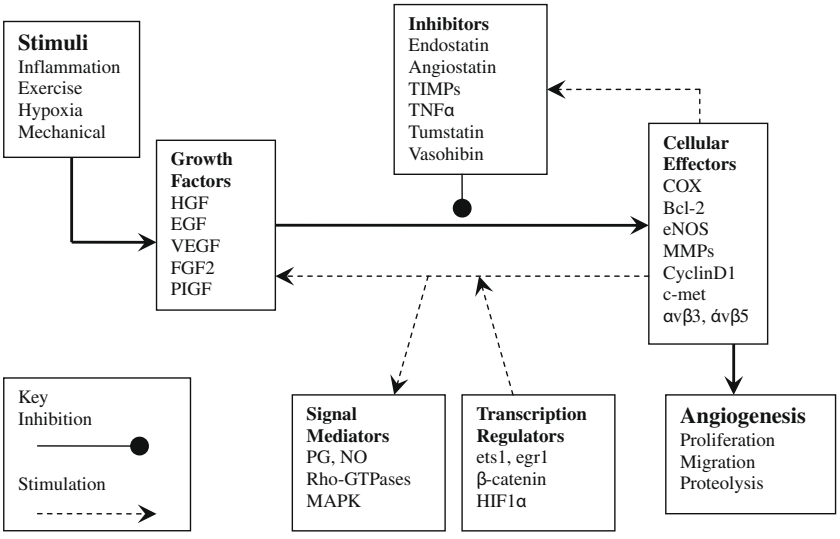

Fig. 2 – The main mediators involved in the angiogenic cascade.

1.2. Omega-3 FA

Omega-3 FAs ($n-3$) are long-chain polyunsaturated fatty acids with the first double bond 3 carbons from the methyl end of the chain. Omega-6 ($n-6$) fatty acids have a similar structure with the first double bond 6 carbons from the methyl end of the chain. Humans are unable to desaturate the $n-3$ or $n-6$ double bond and as such this makes both compounds 'essential fatty acids' obtained only from dietary sources.

Omega-6 fatty acid is consumed as linoleic acid or arachidonic acid found in meats, and vegetable oils (safflower, corn and soybean oil). The principal dietary source of omega-3 FA is from oily cold-water fish namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Both omega-3 and omega-6 fatty acids are used as substrates for the production of eicosanoids that are a class of compounds including prostaglandins (PGs), thromboxanes and leukotrienes intimately involved in immunomodulation, inflammation and tumour formation. Eicosanoids produced using omega-6 fatty acids (arachidonic acid) as a substrate stimulate inflammation and tumour angiogenesis, whereas eicosanoids produced from omega-3 fatty acids, EPA and DHA are anti-inflammatory and do not stimulate angiogenesis.[9,10] Fig. 3 illustrates the basic metabolism of omega-3 and omega-6 fatty acids.

The focus of this review is on the role of these omega-3 FAs as angiogenesis inhibitors and their potential for use as natural chemo-preventative agents at all stages of the angiogenic cascade is examined. Table 1 summarises the evidence for this review.

1.3. Omega-3 FA and VEGF

Vascular Endothelial Growth Factor (VEGF) is a heparin-binding homodimeric glycoprotein with a molecular weight of 45 kDa[11] and a cysteine knot motif shared by other growth factors such as Platelet-Derived Growth Factor (PDGF).[12] The VEGF family comprises five molecules such as VEGF-A, B, C, D and Placenta Growth Factor (PIGF). Each molecule has numerous isoforms of which VEGF-165 was reported to be the most abundant and mitogenic isoform of VEGF-A.[6]

VEGF is a principle factor involved in almost every stage of sprouting angiogenesis, it increases vascular permeability,[13] induces endothelial cell proliferation and migration and promotes endothelial cell survival.[14]

Numerous studies have demonstrated that VEGF or its receptors are up-regulated in many human cancers,[15–22] and omega-3 fatty acids have been shown by a variety of different studies to suppress VEGF production.

1.4. In vitro studies

Human umbilical vein endothelial cells (HUVECs) treated with conjugated EPA showed less VEGF-stimulated tube formation during sprouting angiogenesis than controls, VEGF-stimulated migration of HUVEC was suppressed and certain matrix metalloproteinases (MMPs) associated with endothelial cell migration were diminished in HUVECs treated with conjugated EPA.[23]

A shark oil-olive oil blend inhibited VEGF binding to its receptors (flk-1 and flk-2).[24]

Pre-treating bovine aortic endothelial cells (BAE cells) with docosapentanoic acid (DPA) (an elongated metabolite of EPA) suppressed endothelial cell tube-forming activity induced by VEGF. DPA pre-treatment also suppressed the migratory activity of BAE cells and VEGF receptor-2 expression both in plastic dish and in collagen gel cultures.[25]

A study investigating the effect of EPA on VEGF-induced endothelial cell proliferation using bovine carotid artery endothelial cells (BCE cells) showed that BCE cells treated with 0.5 μg/ml EPA for 48 h displayed a dose-dependent sup-

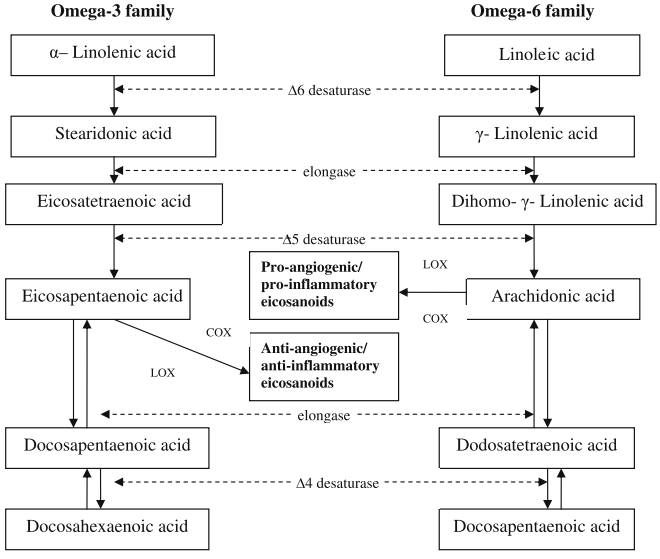

Fig. 3 – Simplified metabolism of omega-3 and omega-6 fatty acids. Basic metabolism of omega-3 and omega-6 FA. Omega-3 FAs give rise to generally antiinflammatory, anti-proliferative mediators, omega-6 family gives rise to more proinflammatory pro-proliferative mediators. COX = cyclo-oxygenase, LOX = lipoxygenase.

Table 1 – Summary of the observed effects of omega-3 FAs on various mediators of the angiogenic cascade.

Mediator	Effect of omega-3 FA
VEGF *in vitro*	Omega-3 FAs suppress VEGF-stimulated endothelial cell proliferation, migration and tube formation during sprouting angiogenesis[23,25,26] Decrease expression of the VEGF receptors flk-1 and flk-2 and inhibit binding of VEGF to its receptors[24,26] Acting upstream inhibit critical mediators in the PGE2-induced signalling pathway which leads to augmented VEGF expression in colon cancer cell lines[27]
In vivo	Nude mice with omega-3 pre-supplemented diets undergoing implantation of human colorectal and breast carcinomas demonstrated a decreased tumour microvessel density and tumour volume compared to controls.[28,29] Amounts of VEGF, PGE2 and COX-2 expressed in these tumours were also decreased[28] Flank implantation of fibrosarcomas in Fischer 344 mice also demonstrated a decreased tumour cell volume and decreased amounts of VEGF-alpha mRNA in those with EPA supplemented diets.[30]
Human	Volunteers assigned a Mediterranean diet with a high omega-3 FA content compared to volunteers consuming an ordinary Swedish diet demonstrated an increased omega-3:omega-6 ratio by 45% with circulating levels of VEGF falling by 13%[31]
PDGF *in vitro*	Inhibit production of PDGF-like protein from vascular endothelial cells[40] Inhibit vascular smooth muscle proliferation by modulating various steps of the PDGF signal transduction pathway[43]
Ex vivo	Quiescent human mononuclear cells from humans with a pre-supplemented omega-3 rich diet expressed reduced amounts of PDGF genes[41]
Human	High dose oral fish oil supplementation of human volunteers yielded a rise in cellular omega-3 levels and significantly decreased levels of PGDF-A and PDGF-B mRNA expression when compared with those on a control diet[42]
PD-ECGF	Little information available. One study demonstrated no change in PD-ECGF gene expression on quiescent human mononuclear cells after prior dietary supplementation with omega-3 FA[42]
FGF	Little information available. Inhibitory effect of omega-3 FA on FGF-induced angiogenesis not observed.[62,63]
HGF, EGF	Currently no studies investigating the effect of omega-3 FA on HGF or EGF. Opportunity for original research
Nitric oxide *in vitro*	Inhibit nitric oxide-dependent angiogenesis in a variety of ways: Inhibit NO production and inducible nitric oxide synthase (iNOS) expression in murine macrophages.[79–82] Down-regulate iNOS COX-2 and TNF-alpha genes by blocking NFKB and MAP-kinase activation[78]
In vivo	A small animal model with endogenously high levels of omega-3 FA demonstrated that the incidence and growth rate of experimentally induced colon tumours were decreased alongside the levels of iNOS and NFKB[84]
COX-2 *in vivo*	Several small animal models have identified that omega-3 FA enriched diets have inhibitory effects on COX-2 and prostaglandin production[27,95] Synergistic inhibitory effects on the growth of experimentally induced tumours of cells from varying human cancer cell lines treated with omega-3 FAs and COX-2 inhibitors have recently been demonstrated[96–98]
MMPs and Beta-catenin *in vitro*	Matrix metalloproteinase 2 and 9 mRNA production is reduced by omega-3 FA.[23] Beta-catenin has also been shown to be reduced by treatment with omega-3 FA[101]

pression to VEGF-induced endothelial cell proliferation. This effect was not observed with BCE cells treated with arachidonic acid or DHA. Flk-1 expression was also inhibited in a dose-dependent fashion in EPA-treated BCE cells.[26]

EPA and DHA inhibited ERK-1 and 2 phosphorylation and HIF-alpha protein over-expression (critical steps in the Prostaglandin-E2 (PGE2)-induced signalling pathway leading to augmented expression of VEGF in colon cancer cells). EPA showed greater efficacy than DHA *in vitro*.[27]

1.5. *In vivo studies*

Omega-3 enriched diets decreased the amount of microvessels developing in HT-29 cell human colorectal tumours implanted in nude mice. The amount of VEGF, cyclooxygenase 2 (COX-2) and PGE2 expressed in the tumours was also decreased.[27] Experiments in which breast carcinomas were implanted into nude mice that were then fed with diets high in EPA or DHA and compared to controls indicated that both tumour microvessel density counts and levels of VEGF measured in the resected tumours were significantly lower in the animals receiving these omega-3 FAs.[28,29]

Fischer 344 rats (200–250 g) underwent flank implantation of the methylcholanthrene-induced fibrosarcoma and were assigned to diets supplemented with corn oil, normal saline or EPA. After resection of the tumour rats with the EPA supplemented diet had a significantly decreased tumour volume

and levels of VEGF-alpha mRNA were also significantly diminished in this group.[30]

A study investigating the effects of a diet high in omega-3 FA (Mediterranean diet) on healthy volunteers found that after 6 weeks the omega-3:omega-6 ratio had increased in those on the Mediterranean diet and levels of circulating VEGF had subsequently decreased.[31]

1.6. *Platelet-Derived Growth Factor (PDGF)*

Platelet-Derived Growth Factors have mitogenic and chemoattractant properties for vascular smooth muscle cells[32] and also stimulate motility of mesenchymal cells such as fibroblasts and vascular smooth muscle cells.[33] Platelet-Derived Growth Factors are disulphide-linked homo- or heterodimers consisting of A or B chains,[34] and five isoforms have been reported, namely PDGF-AA, PDGF-AB, PDGF-BB (the most commonly expressed form), PDGF-C[35] and PDGF-D.[36–38] The PDGF receptor (PDGFR) has two subunits PDGFRα and PDGFRβ and exhibits tyrosine kinase activity. PDGF-BB the most abundant of the isoforms exhibits many angiogenic effects including the induction of VEGF[39] and a recent review reports interest in developing a PDGF/VEGF antagonist as an angiogenesis inhibitor.[7]

In 1988 Fox and DiCorleto demonstrated that fish oils inhibit *in vitro* production of PDGF.[40] Much of the experimental work relating to fish oil and PDGF has centred around angiogenesis and atherosclerosis in the cardiovascular system, nevertheless some of the results may be applied to angiogenesis in general. One study assessing quiescent human mononuclear cells *ex vivo* found that prior dietary supplementation with omega-3 fatty acids suppressed the expression of genes for PDGF.[41] In a randomised observer-blinded controlled trial, 14 healthy males were randomised to receive 7 g/d of an 85% oral fish oil supplement, and 7 acted as controls. Omega-3 levels were measured in monocyte phospholipids and were found to rise in the fish oil group. PDGF-A and PDGF-B mRNA expression in monocytes was measured using polymerase chain reaction (PCR) and it was found that mRNA expression decreased for both PDGF-A (–66%), and PDGF-B (–70%) in the fish oil group.[42]

Omega-3 FAs EPA and DHA have been shown to inhibit vascular smooth muscle proliferation (a component of angiogenesis) *in vitro*, the effect of EPA on the PDGF signal transduction pathway was also investigated. EPA was found to inhibit PDGF binding on its receptor and activation of protein kinase C. EPA also suppressed c-fos mRNA expression, one of the early genes involved in PDGF signal transduction, through partially inhibiting c-fos transcription. The data suggest that EPA may inhibit vascular smooth muscle cell proliferation by modulating various steps of the PDGF signal transduction pathway.[43] In addition, EPA and DHA significantly inhibited PDGF-induced migration of vascular smooth muscle cells *in vivo*.[43]

1.7. *Platelet-Derived Endothelial Cell Growth Factor (PDECGF)*

Platelet-Derived Endothelial Cell Growth Factor or thymidine phosphorylase (TP) was isolated from platelets in 1987,[44] cloned in 1989[45] and identified as a thymidine phosphorylase in 1992.[46] PD-ECGF has been shown to induce angiogenesis in a rat sponge model and in a rat freeze-injured skin model and to cause an increase in tumour growth in breast cancer xenografts transplanted into mice.[47] TP is also known to be induced in several carcinoma cell lines within 6 h by inflammatory cytokines such as TNF alpha, interleukin-1 and interferon gamma and induced up to 47-fold by synergistic action of all three underpinning the carcinogenic effects of some cytokines associated with inflammation.

PD-ECGF is reported to act synergistically in inducing angiogenesis alongside VEGF in gastric cancer.[48] Studies by Takahashi et al. and Takebayashi et al. have investigated PD-ECGF expression and microvessel count[49,50] in 163 colorectal primary tumours reporting that there was an increased microvessel count in PD-ECGF-positive tumours. Furthermore, those tumours expressing PD-ECGF had a highly statistically significant association with tumour size, extent of invasion, lymph node metastases and lymphatic and venous invasion.[50] Of 40 pancreatic adenocarcinomas studied using immunohistochemistry, 30(75%) were said to express PD-ECGF and 27(67.5%) expressed VEGF. In those tumours that expressed both of the above-mentioned growth factors, a higher intertumoural microvessel density was observed indicating increased angiogenic activity.[51]

There is little data assessing the effect of omega-3 in relation to PD-ECGF in angiogenesis. One study using quiescent human mononuclear cells that have been shown to express highly specific mRNA for growth factors demonstrated that there was no change in PD-ECGF gene expression after prior dietary supplementation with omega-3.[42] The effect of omega-3 on the angiogenic activity of PD-ECGF is therefore yet to be investigated and represents an opportunity for original research.

1.8. *Fibroblast Growth Factor*

Fibroblast Growth Factor refers to a family of 20 molecules including acidic FGF and basic FGF, FGF-1 and FGF-2, respectively, with both being implicated in angiogenesis[52] and acting as ligands for tyrosine kinase receptors.[53]

In 1977 FGF was shown to initiate DNA synthesis and proliferation of bovine vascular endothelial cells *in vitro* in concentrations as low as 1 ng/ml.[54] Fibroblast Growth Factors were also shown to be highly mitogenic in rodent, porcine and human granulosa cells.[55]

Later experiments using a sophisticated 3-dimensional collagen matrix for endothelial cell culture demonstrated that FGF-2 greatly increased tubulogenesis of unstimulated human umbilical vascular endothelial cells. FGF-2 was also found to have an additive effect with VEGF, and a synergistic effect in conjunction with a cocktail of nine angiogenic factors. The effect was also noticed in isolation for VEGF, HGF (Hepatocyte Growth Factor or Scatter Factor) and Epidermal Growth Factor (EGF).[56]

Fibroblast growth factors are implicated as tumourogenic factors in a number of human cancers including lung, prostate, pancreas and colon,[57–60] and indeed fibroblast growth factor is associated with an increased risk of metastasis in colon cancer.[61] There is little information on the effect of

omega-3 on FGF but two *in vitro* studies suggest that omega-3 FAs do not have an inhibitory effect on FGF-induced angiogenesis.[62,63] Further investigations into the effect of omega-3 FA on this potent angiogenic factor are required.

1.9. *Hepatocyte Growth Factor*

Hepatocyte Growth Factor/Scatter Factor is secreted from mesenchymal derived cells as an inactive precursor which is activated by urokinase or tissue plasminogen activator. The receptor for HGF is found on endothelial cells and is termed *c-met*.[64] Partly through its own actions and also through its ability to activate VEGF, HGF has been shown to have a strong role in angiogenesis.[65] As yet there are no studies investigating the effects of omega-3 on HGF.

1.10. *Epidermal Growth Factor*

Epidermal Growth Factor binds to Human Epidermal Growth Factor Receptors 1–4 (HER1–4).[66] Over-expression of HER2 in cancer cells is associated with increased VEGF and angiogenic activity via increases in protein synthesis of Hypoxia Inducible Factor 1α (HIF 1α).[67] EGF and HER receptors are associated with the pathogenesis of a number of different cancers including breast, colorectal and pancreatic carcinomas[68–70] and with the promising results from the development of HER receptor antagonists, for example, the anti-HER2 therapy trastuzumab developed for metastatic breast cancer and the development of EGFR antagonists for colorectal cancer.[71,72] No studies have assessed the role of omega-3 fatty acids on EGF or HER.

1.11. *Nitric oxide*

Nitric oxide, produced by nitric oxide synthases, has both vasodilatory and pro-angiogenic effects. It promotes endothelial cell survival, inhibits apoptosis and enhances endothelial cell proliferation.[73,74] Inducible nitric oxide synthase (iNOS) and COX-2-dependent angiogenesis are modulated by VEGF in human colorectal cancer[75,76] and in turn VEGF-mediated angiogenesis is also dependent on nitric oxide production.[77] Fig. 4 illustrates a proposed pathway for increased VEGF production in response to increased levels of iNOS and COX-2.[76] Omega-3 FAs have been shown to inhibit NO-dependent angiogenesis in a variety of ways.

The omega-3 fatty acid alpha-linolenic acid (ALA) has been shown to down-regulate iNOS, COX-2 and TNF alpha gene expression by blocking Nuclear Factor Kappa Beta (NFKB) and MAPK activation in LPS-stimulated RAW 264.7 cells.[78] Omega-3 FAs in particular DHA inhibit NO production and iNOS expression in stimulated murine macrophages.[79–82] Inducible NO and NFKB have been shown to be down-regulated in human colorectal cancer cells treated with DHA.[83] A recent study using a fat-1 transgenic mouse model with endogenously high levels of omega-3 FA demonstrated that the incidence and growth rate of colon tumours (experimentally induced by inflammation and carcinogens) was decreased as were the levels of iNOS and NFKB.[84]

1.12. *COX-2 and PGE2*

Cyclo-oxygenase 2 is an enzyme catalysing the conversion of arachidonic acid (omega-6 fatty acid) into prostaglandins such as PGE2. In general metabolites of omega-6 fatty acids are associated with increased levels of inflammation and tumour angiogenesis.[9,10] Dating back to 1974 both *in vitro* and *in vivo* studies have demonstrated a link between prostaglandins and cancer in particular the E series prostaglandins.[85,86] NSAIDs (COX-2 inhibitors) such as celecoxib have been shown to significantly reduce tumour formation in animal models, and significantly reduce colonic polyp burden by 30% in controlled trials in those with Familial Adenomatous Polyposis (FAP).[87–89] A large nested case-controlled study found that long-term NSAID/COX-2 inhibitor usage was associated with a significantly decreased risk of developing colorectal cancer.[90]

COX-2 is up-regulated in most human cancers[75,91] and PGE2 is produced in large amounts in colorectal tumours and has been shown experimentally to induce the production of pro-angiogenic factors in many cell types.[92,93] A recent study by Cianchi et al. revealed a stimulatory effect of nitric oxide on COX-2 activity in human colorectal cancers[76] furthermore, this interaction is likely to yield a co-operative effect in promoting angiogenesis through a PGE2 increase in VEGF production.[94] Several small animal models have identified omega-3 fatty acid-enriched diets as having inhibitory effects on COX-2 and prostaglandin production in both plasma and experimentally induced tumours. Rats fed with a corn-oil diet (rich in omega-6) or a flaxseed oil diet (rich in omega-3) were subject to chemical induction of colon tumours. Tumour

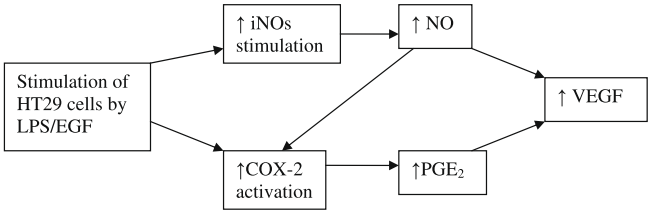

Fig. 4 – Potential mechanism of iNOS and COX-2 pathways in stimulating VEGF production proposed by Cianchi and colleagues.[76] Experimentally stimulated HT29 colon cancer cells are stimulated by LPS or EGF and in turn cause stimulation of inducible nitric oxide synthase (iNOS) and increase the production of cyclooxygenase-2 (COX-2). iNOS production stimulates NO production which causes increased production of both VEGF and COX-2. Increased COX-2 activation stimulates further production of PGE2, which also increases VEGF production.

incidence was decreased in the flaxseed oil (*n* – 3) group compared to the corn oil group (*n* – 4) (29.4% versus 82.6%) and levels of COX-1 and COX-2 were significantly reduced in the flaxseed oil group.[95] The effect of EPA and DHA on human colorectal cancer cell lines both *in vitro* and *in vivo* upon tumours transplanted into nude mice has also been investigated. EPA and DHA reduced VEGF, COX-2 expression and PGE2 levels in HT-29 cells cultured *in vitro*. EPA and DHA also inhibited ERK-1 and -2 phosphorylation and HIF-1alpha protein over-expression, critical steps in the PGE2-induced signalling pathway leading to the augmented expression of VEGF in colon cancer cells. EPA and DHA also reduced growth of tumours obtained by inoculating HT-29 cells in nude mice, microvessel formation and the levels of VEGF, COX-2 and PGE2 expressed in tumours.[27] Recent evidence reveals a synergistic inhibitory effect on the growth of experimentally induced tumours or cells from varying human cancer cell lines treated with omega-3 FA and COX-2 inhibitors.[96–98] Hypoxia Inducible Factor (HIF) serves as a pro-angiogenic factor acting upstream from VEGF. HIF 1α has been found in a number of human cancer cell lines and is associated with *in vitro* tumour vascularisation.[99] HIF 1α has been identified as a pivotal transcription factor linking the inflammatory and oncogenic pathways via Nuclear Factor Kappa Beta, COX-2 and PGE2 mechanisms.[100]

1.13. *Matrix metalloproteinases*

Matrix metalloproteinases are zinc-dependent proteases which have a critical role in the proteolysis of the basement membrane – a key phase in sprouting angiogenesis. Certain MMPs produced by endothelial cell are also involved in capillary sprouting.[64] MMPs 2 and 9 mRNA production was shown to be inhibited by conjugated EPA in a study investigating the effect of conjugated EPA on VEGF-induced angiogenesis in human endothelial cells.[23]

1.14. *Beta-catenin*

The production of this transcriptional regulator in the angiogenic cascade has been shown to be inhibited in colon cancer cells treated with DHA.[101] Several other proteins regulated by the TCF-beta-catenin pathway and involved in regulation of tumour growth and angiogenesis were also down-regulated by DHA, including peroxisome proliferator-activated receptor delta, membrane type 1 (MT1)-matrix metalloproteinase (MMP), MMP-7 and VEGF.[102]

2. Conclusion

In 1863 Rudolf Virchow described the relationship between inflammation and cancer when he observed leucocytes in neoplastic tissue.[8] Today it is accepted that chronic inflammation is a predisposing factor for many human cancers such as Barrett's oesophagus and its association with adenocarcinoma of the oesophagus.

Factors such as PGE2, nitric oxide, COX-2 and NFKB have well-documented roles in both the inflammatory and angiogenic cascades with significant cross-relation in both pathways and this review demonstrates the potential for omega-3 FAs as anti-inflammatory and anti-angiogenic agents via inhibition of these factors and others including VEGF and PDGF.

With the development of safe parenteral preparations containing significant amounts of omega-3 FAs, human trials have demonstrated that parenteral administration of fish-oil lipid emulsion leads to a significant and rapid increase in EPA and DHA concentrations in plasma, platelet and leucocyte membrane phospholipids (within hours)[103–105] this was previously not achievable with oral preparations. These trials have also suggested that omega-3 FAs via their immunomodulatory effects decrease the re-operation rate,[106] the requirement for post-operative antibiotics,[107] the rate of sepsis,[108] the incidence of post-operative venous thromboembolism[104] and the length of hospital stay,[106,109] and lower the mortality rate for surgical patients.[108]

This development of commercially available human parenteral infusions of omega-3 fatty acids offers perhaps the greatest opportunity to date for modulating immune function in the chemoprevention of cancer whilst patients experience the myriad of other beneficial effects associated with parenteral omega-3 FAs. Randomised controlled trials assessing the effects of parenteral omega-3 fatty acid administration in human cancer patients are now awaited with interest.

Conflict of interest statement

Mr. A. Dennison, Miss. L. Spencer, Miss. M. Webb, Mr. C. Mann, Mrs. C. Pollard and Mr. M. Metcalfe are investigators in a trial using omega-3 FA as potential angiogenesis inhibitors in human hepatic colorectal metastases. This trial has received funding from B. Braun Pharmaceutical Company. Mr. D. Berry, Professor W. Steward and Mr. D. Spencer have no conflict of interest declared.

Acknowledgements

We would like to thank Professor P.C. Calder, Professor of Nutritional Immunology, Institute of Human Nutrition, University of Southampton School of Medicine for his suggestions regarding this manuscript. We would also like to thank Mrs. P. Divall, clinical librarian at Leicester General Hospital for her help with literature searching and obtaining original manuscripts.

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