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Omega-3 Fatty Acids Can Improve Radioresponse Modifying Tumor Interstitial Pressure, Blood Rheology and Membrane Peroxidability

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Abstract. *Several studies provide evidence that hypoxic cells present in animal and human solid tumors, may be critical for the successful treatment of cancer. In particular hypoxic cells are resistant to ionizing radiation, photodynamic treatment and the large majority of chemotherapeutic drugs. Hypoxia is generally due to the inadequacy of vascular beds supporting the tumor and to an abnormal microcirculation. Three parameters, tumor interstitial fluid, hemorheological factors and lipoperoxidation, are considered and tentatively associated as playing a role in hypoxic cell treatment. Omega three fatty acids modify these factors and are discussed for their possible ability to enhance tumor cells susceptibility to radiotherapy.*

Chronic and transient deficiency of blood flow inside the tumor mass result in the development of the so-called chronic or acute hypoxia, respectively (1). Chronic hypoxia or diffusion limited hypoxia is thought to develop because the tumor proliferation is so rapid that the vasculature cannot develop at a corresponding rate. Tumor cells close to capillaries are well oxygenated and rapidly dividing, but as the oxygen tension decreases with distance from the nutritive capillary they become less active and some die. Deprivation of oxygen and nutritive substances is responsible for the areas of necrosis which usually develop about 150-200 μm from the nearest blood vessel (2). Acute or transient hypoxia is caused by a temporary interruption or lowering of the blood flow through the individual vessels within the tumor. It could be explained by: (a) a time-dependent compression of the expanding tumor parenchyma on thin walled capillaries; (b) interstitial fluid pressure increase and (c) microcirculatory disturbances including hemorheological disfunctions and thrombosis (3,2). Recent histochemical studies on animal tumors with fluorescent and enzyme probes have provided indirect evidence of this phenomenon and shown that inside the tumor mass the two kinds of hypoxia coexist and create a microevironmental heterogeneity (4). Although the hypoxia mechanism may be different and variable according to the tumor growth, the resultant hypoxic cells at the interface region between the well oxygenated tissue and the necrotic areas can remain potentially viable and clonogenically active for a considerable period. Their energy is dependent on anaerobic glycolysis and their proliferation halts in the GO/G1 cycle phase, a radioresistant state (5-7). The reoxygenation of this hostile microenvironment results in endothelial injury and can select cells more aggressive and resistant to drugs. The rationale for such resistance is still under discussion. It seems independent of the presence of P-glycoprotein and correlated with the amplification of some genes as shown by the enhanced dihydrofolate activity (8,6,7,9-11).

Numerous strategies have been attemped to increase tumor blood flow and oxygenation, thereby enhancing the effects of chemotherapy and radiotherapy. The approaches aimed at increasing the oxygen carrying capacity of blood have some technical difficulties, therefore new drugs acting on blood flow have been proposed (12-15). Only recently Essential Fatty Acids (EFAs) have been tested in radiotherapy principally oriented to radioprotection (16,17). This review will deal with some of the generating mechanisms of hypoxia and with the various possibilities offered by w-3 on tumor growth, oxygen supply and radiosensitization.

Tumor interstitial fluid (TIF) formation and pressure

Gullino has reported that tumor growth in associated with the accumulation of a large volume of fluid (TIF) inside the tumor interstitium. This occupies from 30% to 60% of the tumor volume and has a different biochemical nature compared to normal fluids (18,19). TIF accumulation and pressure are the net results of various pathophysiological mechanisms: (a) secretion of Vascular Permeability Factor by tumor cells, (b) waste metabolites, (c) increased transcapillary filtration, (d) lymphatic drainage absence and (e) inflammatory reaction at the tumor-host periphery

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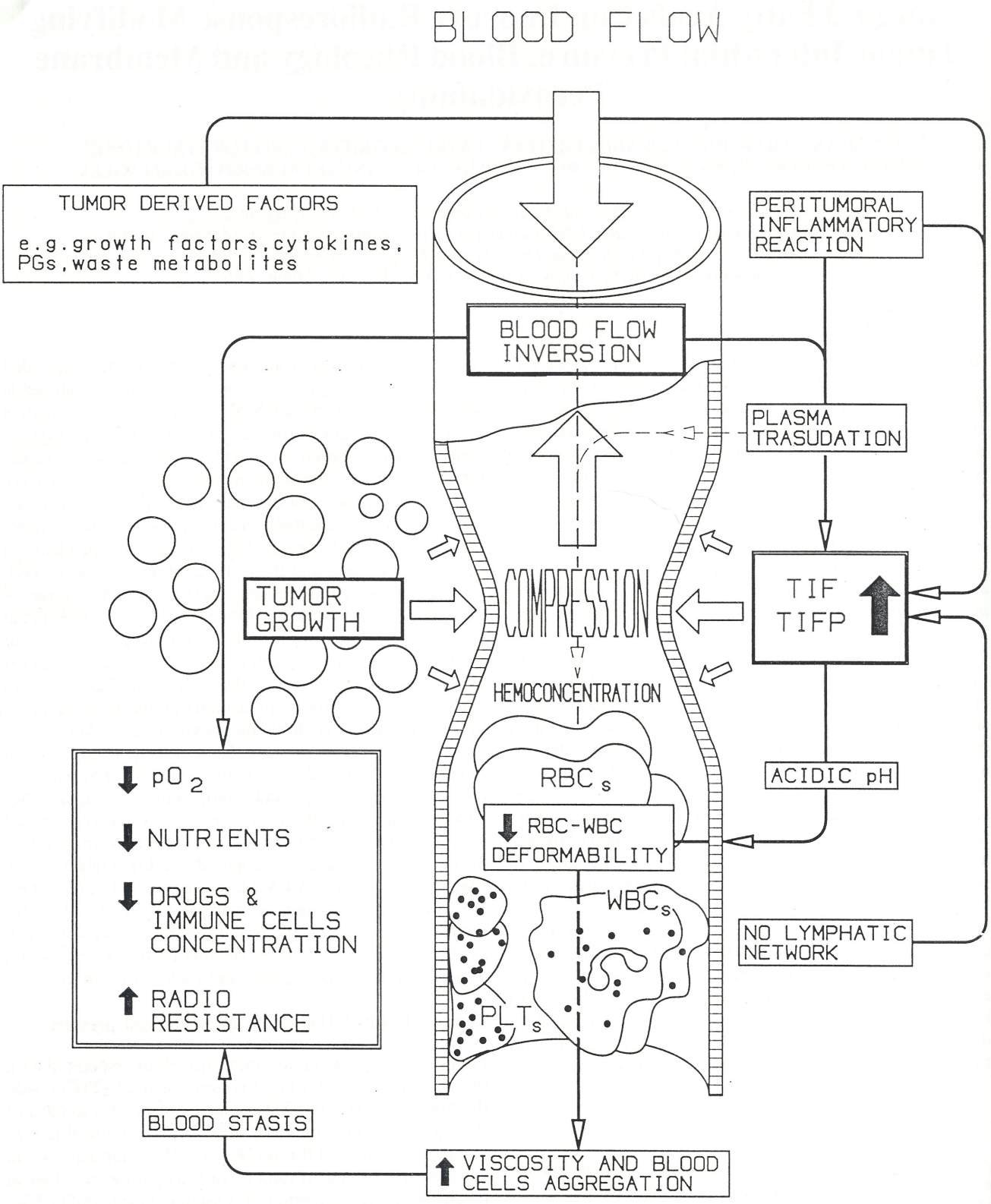

Figure 1. *The various processes which lead to an anomalous tumor microcirculation are illustrated together with the effects on different substances accumulation and response to treatment.*

(19-22). The decreased outflow of this fluid generates an increased TIF pressure which, combined with the pressure of the expanding tumoral tissue in a confined space, determines a temporary occlusion of capillaries. This leads both to an increases of blood pressure on the arterial side of the capillary bed and to an increased transudation and edema formation as schematically seen in Figure 1 (23-24). Interstitial fluid pressure has been measured in human and experimental tumors: it was significantly higher than in normal tissues. A mean increase of 23.9 mmHg, with a maximum of 50 mmHg, has been measured by Jain and coworkers on colon, breast, head-neck carcinomas and metastases specimens from lung, liver and lymph nodes (25, 26). TIF pressure increased from the periphery to the center of the tumor and was sufficient to cause blood vessel collapse (27). The impaired local blood flow resulting from this compression evokes tissue hypoxia, even in a well perfused zone of tumor mass. Hypoxia is compensated by an increase in anaerobic glycolysis which lowers the TIF pH (18,28). The inflammatory reaction at the tumor periphery perticipates in TIF formation and constitutes part of a complex phenomenon that contributes to the outcome of highly heterogenous genotypes and selection of subpopulations better fitted to metastasize (21,22). It has all the histological hallmarks of a chronic inflammation and resembles a wound which does not heal. The various mediators, locally produced *(e.g.* histamine, serotonine, prostaglandins/leukotrienes, Platelet Activating Factor, Platelet Derived Growth Factor, cytokines, growth factors) modulate mesenchymal cell proliferation and particularly angiogenesis, making the tumor endothelium even more leaky (20,29). The latter effect associated with the accumulation of collagen and mucopolysaccharides and products of matrix proteolityc degradation (charged macromolecules) create a Donnan effect in favor of TIF accumulation (18,30). The convective streams of TIF (directed from the tumor center towards the periphery) and the increased vascular-tumor distance thus produced reduce oxygen diffusion, and drug distribution and enable tumor cells to invade host tissues along paths of less resistance. The increased and nonhomogeneous cellular water content and tumor interstitial compartment, compared to the vascular compartement, gives an idea of the lenght that must be covered by a molecule to reach the tumor and the dilution effect to which is submitted (19).

Tumor blood flow and rheology

Tumor vasculature consists of two different population: pre-existing host vessels incorporated into the tumor tissue and newly induced microvessels (2). The host vessels are the only ones able to respond to physiological and pharmacological stimuli and do not grow proportionally to the tumor mass increase. This leads to a decreased diffusive area for nutrients, wastes and oxygen (31). Spontaneous vasomotion is mostly absent in tumor arterioles, which often appear permanently dilated. Cancer vessel architecture is chaotic and irregular with arteriovenous shunts not participating in microcirculatory exchange (32,33). Hypoxic and ischemic microareas coexisting with high vascularized areas imply a functional disturbances of the macro and microflow (34, 32, 35, 36, 37).

Blood flow rate in most studies on rodent tumors decreases with tumor size when compared to normal tissue. However, in the minority of experiments such decreased flow has not been confirmed even in a similar tumor type. Several pathophysiological mechanisms have been proposed for explaining this difference: transplantation site, time of tumor growth, flow registration and recording methods (13). Blood flow measurements in human cancer show heterogenous values included between highly vascularized organs such as brain and poorly vascularized such as adipose tissue. Perfusion flow at tumor level is higher or lower than in the tissue of origin, depending on the physiological state of the latter. It is higher at the tumor periphery than on its central zone and in general primary tumors are better supplied than metastatic lesions (31).

The major factors which determine tumor blood flow are the pressure difference (perfusion pressure) and the resistance to the flow across the vascular bed (26). Studies on tumor perfusion pressure, even if limited, indicate an arterial pressure similar to that on normal vessels and a significantly decreased venous pressure (34,12). Two possible reasons have been put forth: an increase geometric (z) and flow resistance (34,38). Experimental studies on tissue - isolated mammary adenocarcinoma (R3230AC) and on P22 carcinosarcoma have shown that z increases with the tumor volume and vessel tortuosity and varies with the location of tumor transplant (39,33). Blood viscosity in tumors is increased and correlated with the shear rate and the hematocrit. The hematocrit inside the tumor microcirculation becomes 5% higher than in systemic circulation, probably due to plasma extravasation (24, 36). This hemoconcetration is associated with a less pronounced Fahraeus - Lindquist effect and with a decreased blood cell deformability (34, 40).

Decreased Red Blood Cell (RBC) deformability measured by filtration method, has been found in mice transplanted with Lewis carcinoma and in other tumor models (34,41). The acidic pH in the hypoxic areas further contributes to decreasion RBC membrane deformability (42). This factor, together with the increased fibrinogen binding to erythrocyte surface, leads to a mechanical trapping of RBCs inside the tumor microcirculation. The flow slowdown that follows and the plug of less deformable leucocytes and platelets trigger a vicious circle that perpetuates sludging, stasis, hypoxia and thromboembolism (see Figure 1) (43,36). Moreover the stiffer RBC membrane decreases its surface of exchange with the endothelium and consequently the O_2 diffusion to tissue (43,28,44).

The inflammatory reaction, with local production of

cytokines *(e.g.* TNF) can also alter blood flow by increasing vascular resistance, the platelet aggregability and the leukocyte and lymphocyte adhesion to vascular endothelium (21,45,46,47).

The increased fibrinogen - globulin levels and erythrocyte sedimentation rate reported in many patients with bowel, lung, melanoma and breast carcinomas are evidence of the presence of hemorheological abnormalities in human tumor microcirculation (43,34,48). Dintenfass, studying different rheological parameters in 130 melanoma patients, found that they were correlated with survival time and were a sensitive predictor of relapse (43).

In summary, it can be said that acute hypoxia, due to a temporary blood flow impairment, worsens the effect of Tumor Interstitial Fluid Pressure (TIFP) favoring a non homogeneous tumor environment, growth and radioresponse (3).

Tumor lipoperoxidation

Radiation energy exerts its biological effects by a means of direct are indirect effect on target cellular components. The indirect effect, which is responsible for at least two-thirds of all the cell-radiation killing effect, is due to the ionization of cellular and extracellular water molecules, which make up about 70-80% of the tumor mass. The impact of low Linear Energy Transfer (LET) radiation is related to oxygen pressure and to secondary electrons scattered by the interaction of photons with water. The latter effect gives rise to a free radical reaction modulated by superoxide radical O_2, which is pH and O_2 related. When these two parameters are lowered, water radiolysis quickly solves in a back reaction without any biological impact, whereas in (almost) normal conditions of pH and O_2 pressure a radical clustering follows the radiation path and interacts with DNA and other secondary intra and extracellular substrata such as biological membranes (5). The interaction with membrane polyunsaturated fatty acids together with the presence of oxygen and metals (*e.g.* Fe++) initiates a chain reaction that produces a new class of free radicals to a such an extent that it can result in a cell death. This is accomplished by the interaction of peroxidation products with biomolecules of important and regulatory function such as DNA or proteins. Moreover, the oxidation of membrane phospholipids inactivates enzymes and surface transporter proteins, causing changes in water and ion content of the cell (49,50).

Two effects of lipid peroxidation are responsible for this change in permeability: first, an increase in the dielectric constant of the surfaces of inner membranes due to the accumulation of polar compounds and second, a localized increase in microviscosity due to a reduction of lateral movements of lipids with a creation of pore-like structures (50). Hydroperoxides, the primary products of lipoperoxidation, are unstable *in vivo* and able to propagate such a process. The secondary intermediates include stable molecules, such as aldehydes, which can be cytotoxic and antiproliferative (49,51,52). Studies on regenerating liver seem to demonstrate an inverse relationship between lipid peroxidation and DNA synthesis. A possible explanation is that during mitosis the genetic material is uncovered and therefore rather susceptible to peroxidative and free radical damage (53,54). Animal tumor cells have a much lower susceptibility to both spontaneous and forced lipoperoxidation than healthy tissues. This decreased peroxidative activity is inversely correlated with the tumor growth rate and is due to a lower unsaturation of membranes, to a greater tocopherol-oleic acid content, to a decreased content of cytochrome P450 and to a decreased phospholipid content associated with a low concentration of EFAs and occasionally an increased proportion of cholesterol. This alteration is present not only on the cytoplasmatic membranes but also in the membranes of many organelles such as mitochondria (54). By contrast, this unidirectional shift in lipid saturation is not present in all human solid tumors. For example, brain tumors have a greater phospholipid - cholesterol ratio than breast tumors (57). In this latter, an association between phospolipid content and tumor aggressiveness has been demonstrated (58). Other authors have reported an increased concentration of oleic acid (which has antioxidant activity similar to Vit. E) inside the tumor membranes and no changes in other major fatty acids (59). This increased incorporation is due to activation of 9-desaturation enzymes induced by the lack of EFAs supplement to dividing cells and to a constitutional impairment in Delta-6 desaturase activity in malignant cells (60,61).

Emanuel, studying by Electron Spin Resonance (ESR) the spontaneous generation of free radical inside the tumor mass, found a temporary and a spatial distribution dependent on tumor vascularization. The maxium radical formation rate was present at the peripheral zones of the tumor but had fallen in the central necrotic portion (12). In these low perfused zones Freitas *et al* have proved the accumulation of neutral lipids (triglycerides and cholesteryl esters) in the membranes and in the interstitium as lipid droplets (29).

Another aspect of radiation-membrane interaction is the release of arachidonic acid and its main metabolites. Their elevation is time and organ related and responsible for either toxic or protective effects (17).

Therapeutic opportunities offered by omega three fatty acid

Omega three fatty acids (w-3) are a class of polyunsaturated fatty acids (PUFAs) of marine origine which offer a new opportunity in cancer therapy treatment (63). These PUFAs have alpha linolenic acid as chief family member; their first double bond is located between the 3rd and 4th carbon atom along the fatty chain, starting from the methyl end of the molecule (64). Humans are not able to introduce a double bond at this position, although fish and

plants can do so. These fatty acids are Essential Fatty Acids (EFAs) because they are not synthesized de novo and therefore are ultimately derived from the diet. The w-3 are contained in the diet mainly in fish oils. Water vegetation, algae and phytoplankton can synthesize the first member of this series, linolenic acid (18:3 w-3), and fish feeding on these convert linolenic acid to the two most abundant components Eicosapentaenoic [EPA] (20:5 w-3) and Docosahexaenoic acid [DHA] (22:6 w-3) (65).

Tumors require fatty acids as oxidative substratum, to repair the membrane lipid components and for the synthesis of new membranes needed for growth and cell division. The fatty acids necessary for this purpose are mainly opbtained either by synthesis from glucose or can be supplied by the host as free fatty acids; but they can also be derived from triaglycerols contained in plasma lipoproteins. Tumor synthesis is reduced when an adequate supply of circulating fatty acids is available from the host as diet (66). Therefore, neoplastic cells when supplied with w-3 incorporate these lipids into their membranes, altering their physical and functional properties (64). *In vitro,* 72 hs at least are necessary to completely change the tumor membrane phospholipids. *In vivo,* for example in leukemia, the plasma membrane changes are evident after three weeks of diet and reach a plateau after five weeks (65). In experimental solid tumors the variation in the diet composition also affects normal tissues such as thymus, lymphocytes, platelets, endothelium and bone marrow. These changes have important consequences for the neoplastic and normal cells, influencing tumor growth, membrane peroxidability, transport of substance, receptor -interaction, blood and tumor cells membrane fluidity, platelet aggregation and therapeutical modalities *(e.g.* drug uptake, hyperthemia) (65,67).

Effects of w-3 on viscosity, platelet aggregation and endothelial interaction. The beneficial role of omega three lipids on hemoreology and hemostasis has recently been demonstrated by a large number of studies. The increase in blood fluidity seems linked to a change in the RBC deformability and plasma viscosity. Kobayashi *et al*, in two studies, have demonstrated a significant decrease in platelet aggregability and an increased erythrocyte deformability after a 4 week daily ingestion of 3.5 g of EPA. This implies that the viscoelastic properties of RBC membrane are changed by the ingestion of w-3, as demonstrated by the filterability method (68). The lipid profile in RBC membranes changes after two weeks of supplementation and the EPA incorporation is greater than that of DHA. This membrane unsaturation is responsible for the increased fluidity and for less hindrance to RBC flow (69). Studies by Ernst on blood viscosity, plasma viscosity, red cell deformabilty and aggregation have shown an alteration on all the parameters with an incresing dose on volunteers and in patients with hyperlipoprotenaemia. In the latter group the effect was due to an increased RBC deformability and to a decrease in some components such as lipoproteins and fibrinogen (70). A reduction of fibrinogen and plasminogen activator inhibitor-1 was found in the blood both of normal subjects and of subjects with a coronary disease submitted to a diet rich in w-3. The effect was dose dependent and seen after at least 4 weeks of supplementation (70).

Platelet-endothelium interaction plays an important role in tumor dissemination and in tumor oxygenation due to thrombus formation and platelet aggregation that temporarily stop the blood flow. In this sense the many facilitating factors existing in the tumor are: (a) the slow blood flow rate (b) the haemoconcentration and (c) the production of coagulative and proaggregatory molecules, such as prostaglandins (PGs), Platelet Derived Growth Factor (PDGF), TNF, Interleukin 1 (IL1) and fibrinogen by tumor and neighbouring endothelium. Tumor cells disrupt the intravascular balance between PGI2 and thromboxane A2 (TXA2) in favor of platelet aggregation. At the same time PDGF, a potent mitogen and growth proliferative factor is produced by platelets. PDGF is synthesized by megakaryocytes, stored in the alpha granules of platelets and released during platelet interaction favoring metastasisation and neoplastic growth. Fibrin and plasminogen activator play a role in tumor stroma formation and therapy in its development and propagation (20). The amount of fibrin varies in each individual tumor and arises by extravasation and coagulation of fibronogen. This fibrin deposition serves as a coating that hinders lymphocytes, macrophages, drugs and inflammatory cells from reaching the tumor area and as an initiator of blood vessel growth (71,72,73).

With w-3 intake, EPA and DHA compete with Arachidonic Acid (AA) incorporation in the membrane phospholipids inhibiting its conversion to prostanoids and thromboxanes of the 2nd series (PGE2, TXA2). EPA is converted to 3rd series prostanoids or 5th series leukotrienes, (e.g. L TB5) which differ in potency with respect to 2nd and 4th series analogues. The reduction of TXA2, with the preserved production of PGI2, supplemented by the presence of PGI3, changes the prostacyclin-thromboxane axis equilibrium in favour of a decreased platelet vascular adhesion. The formation of LTB5 and the descreased production of IL1, TNF and PDGF also reduces the recruitment of neutrophils and macrophages to the inflammatory area and the interaction with the endothelium (74, 75).

Fish oil supplement has proved to increase endogenous production of Tissue Plasminogen Activator and to inhibit its inhibitor. w-3 effects on fibronogen are controversial as are those of other clotting proteins, such as antithrombin-3 (AT3). Some works have shown an AT3 elevation with a supplementation of 10 g of either fish or vegetable oil. A recent work with a low concentration of fish oil intake (2.2 g/day, 17.5% EPA - 19.9% DHA) after 20 weeks of supplementation has demonstrated a medium reduction of fibrinogen level of 22% although other authors have not confirmed this result (76,77,78). Another aggregating factor

produced during inflammatory reaction by leukocytes, monocytes, mast cells and endothelial cells, platelet-activating-factor (PAF), is decreased *in vitro* and *in vivo* by fish oil supplementation (79).

Effects of w-3 on peroxidability and tumor growth. Epidemiological and experimental studies have shown that the composition of dietary fat influences and modulates tumor growth and metastasisation (80,81). Dietary PUFAs as compared to monosaturated ones, are known to enhance the development of some kinds of experimental cancers such as breast, prostate, colon and pancreas (82). Not all PUFAs are involved in this sense; in fact, some omega 6 (Gamma Linolenic Acid (GLA) and w-3 have shown a protective effect against the development and progression of these tumors (83). Different mechanisms have been suggested, although the precise way of action remains unclear.

This kind of fats is able to induce a high toxicity against tumor cells, by increasing lipid peroxidation and generation by lipoxygenases toxic products such as hydroperoxides (HPETES) and hydroxy fatty acids (HETES). Omega 3 and GLA selectively kill prostatic, breast, colon, glioma, melanoma, ascite, fibroblast and lung cancer cells *in vitro,* while normal cells are spared (their division rate is lowered) (84,60,8). Begin *et al* have shown that the supplementation of EFAs and iron increases the killing effect (52). Other reports have confirmed these effects on differerent tumor cells lines and results are synthesized in the reviews by Begin and Cave (84,86,85). Gonzalez and Welsch have demonstrated in a recent work on mammary gland tumorigenesis that the inhibiting process is linked to the accumulation of lipid peroxidation products in the tumor tissue and that the addition of an antioxidant like butylated hydroxytoluene is insufficient to suppress the effect (87). Vitamin E has antioxidant activity and can prevent lipoperoxidation but, as reported by Begin, there is experimental evidence that EFAs concentration can be adjusted to overcome this inhibitory effect (52). The most reasonable mechanisms responsible for the killing effect by w-3 may be the interaction by its metabolites *(e.g* hydroxyhexenal, ethane) with DNA structure or enzymes which regulate its functions. The aldehydes derived from w-3 inactivate DNA polymerases or break DNA stands during the S phase. It is during this period that DNA is uncoiled and so more susceptible to the lipoperoxides effects (49,64). Not all studies agree upon the phase sensitivity; in fact, some authors outline that DHA seems to arrest the sensitive cells in the G1 phase of the cycle (44). These authors also report that DHA was more effective than EPA in inhibiting the proliferation of some human lung adenocarcinomas and gliobastoma *in vitro* (44). Horrobin and Begin have proved that the maximum cancer killing effects are obtained by EFAs with 3,4 and 5 double bonds, whereas fatty acids with 2 and 6 double bonds are less effective. The influence of isomeric form of double bond seems of no importance, whereas the degree of differentiation of tumor cells can change the sensitivity to undergo peroxidation (52,61). These authors conclude that a diet composed of GLA and EPA is the best combination to obtain an inhibitory effect.

Two other mechanisms are modulated by w-3 or Gamma linolenic acid which involve altered prostaglandin metabolism and may control tumor growth out of lipoperoxidation. The first is that tumor cells show high PGE2 levels compared to low levels of PGI2, PGE1 and TXA2. This anomalous profile in PGs causes a decreased cAMP level in tumor cells and an intracellular accumulation of Ca++ with a promotion of tumor growth. The second is that tumors may subvert the immune system in part via PGE receptor density and production. On the one hand an excessive PGE2 content has importance in inflammatory reaction modulation but on the other hand it inhibits the mitogen-induced lymphocyte proliferation, lymphokine production and the tumoricidal activity of macrophages (88,89,90). w-3 have been shown to compete with AA for the cyclo-oxygenase activity and to restore macrophage mediated cytolysis (84,91,60,90).

Effects of w-3 on tumor stroma. The tumor consists of neoplastic cells and stroma. The stroma is a lively structure produced by host cells (macrophages, inflammatory cells, neovessels and connective cells) in response to neoplasia. Different growth factors (Epidermal growth factor (EGF), PDGF, hormones, transforming Growth Factor (TGF), Prostaglandins and cytokines (*e.g.* IL1, IL6, TNF) play a role in its growth (21).

w-3 have been demonstrated to lower significantly the production of PGs, cytokines (IL1, IL6, IL2, GMCSF) and some growth factors (estrogen, Prolactin, EGF, TGF) (92,93,94). Recent studies *in vitro* on melanoma and human fibrosarcoma have also proved that EPA reduces the production of collagenase IV, indicating that the 5-lypoxygenase pathway is involved in the invasive activity of tumor cells (95). Neovascularization and macrophage activity, which have a role in tumor progression and regrowth after radiotherapy, are two other processes that may be controlled by w-3 as reported by some authors (90,96,97).

Conclusion

As reported by many authors, tumors have both micro and macroscopic heterogeneity in their blood flow (1,98). This non-uniform spatial and temporal distribution leads to a decreased and non homogeneous oxygenation of tumor mass and to an increased radioresistance (5,99,3). The oxygen delivery to tumor (DO_2)is the product of oxygen content of the blood (CaO_2) and the volume of blood perfusing the tumor mass (Q). Flow rate is proportional to the perfusion pressure (Artero-venous pressure difference) and the flow resistance, which is the product of geometric resistance and blood viscosity. Since geometric resistance is a complex function of the vascular morphology and cannot be modified, DO_2 could be changed by varying: (a) the perfusion pressure; (b) the arterial O_2 concentration and (c) the blood viscosity (25,34). As outlined by Jirtle and Vaupel, drugs or means which alter the diameter of tumour blood vessels would be

more effective in increasing blood flow than those which change viscosity. However, the tortuous nature of tumor vessels creates an increased intratumor resistance and an anomalous microcirculation to such an extent that small changes in blood viscosity may be amplified, especially at the tumor microcirculatory level (12, 31).

Changes in viscosity or hemorheology may be obtained by: (a) hemodilution; (b) reduction of the concentration of high molecular weight plasma proteins (fibrinogen, globulins) and (c) use of drugs to improve parameters associated with blood rheology (RBC-WBC deformability, red cell platelet aggregation).

Vaupel *et al* have evaluated whether hemodilution can improve tumor blood flow (TBF) and hence oxygen supply to the cancer cells. They studied this effect by two different approaches: (a) localized hemodilution in a tissue-isolated tumor and (b) systemic hemodilution in amimals bearing transplanted sarcomas. Starting from a physiological hematocrit (Ht) value of 0.44 and gradually decreasing this by different methods, they demonstrated an increase in TBF, in O_2 availability and in glucose uptake by tumor cells. In localized hemodilution experiments this inverse relationship was also linear for low Ht, whereas in systemic hemodilution the linearity was valid only for Ht values between 0.44 and 0.36. Systemic hemodilution, a more realistic pathophysiological situation, resulted in a two-fold increase in TBF, compared to the three-fold increase during localized hemodilution. The difference between the two approaches is due to the reaction of normal vessels incorporated inside the tumor to the Ht change (100).

Another way to improve the delivery of oxygen to the tumor is the use of drugs which increase the RBC deformability. The erythrocyte deformability is of special importance in a hostile enveronment such as that of the central portion of a tumor, in which that low oxygen content, the high CO_2, the low pH and the decreased ATP content cannot maintain the normal intracellular levels of calcium and consequently the normal membrane plasticity (12,38).

Two drugs with different characteristics deserve attention: flunarizine, a calcium antagonist, and pentoxifilline (PTX), a xanthine derivative. Flunarizine can ameliorate tumor blood flow, radiosensitivity and drug uptake by modifying blood viscosity, RBC shape and vessel diameter (101, 102). Jirtle has demonstrated that with flunarizine it is possible to decrease acute hypoxia by 40% and about 10% the total hypoxic fraction (12).

PTX not only increases the oxygen delivery, modifying the deformability of red blood cells, but also prevents the aggregation of platelets, makes the leukocytes less sticky and modulates the production of TNF by macrophages. Different studies have shown that PTX can increase oxygen delivery to the tumor at a very small dose and prevent late radiation injury in humans (34,103,104,105).

Another molecule to discuss is indomethacin a non steroidal antiinflammatory substance. Milas has pointed out that murine tumors which produce a greater quantity of prostaglandins develop a radioresistance that can be prevented by indomethacin. Moreover, indomethacin decreases significantly the tumor bed effect by modulating angiogenesis and protecting some normal tissues such as bone marrow from radiation (96).

w-3 as demonstrated by different authors, can modify the blood viscosity, the RBC deformability, and the platelet and leukocyte aggregation. It is therefore possible to reduce the temporary stop of tumor blood flow due to the platelets plug. This, associated with the decrease of fibrinogen and other acute phase proteins, may also lessen the rouleaux formation, the RBC sludge and consequently the stasis-hypoxia vicious circle. Furthermore, they modulate, like indomethacin, the macrophage suppressive activity (due to excessive concentration of PGE2) and the production of cytokines and PGs consequently reducing the inflammatory stimuli. Inflammation, thanks to the production of PAF, Leukotrienes and PGE2, increases the vascular permeability and the plasma exudation of tumor capillaries contributing to TIF accumulation (106). TIF together with altered microcirculation is responsible for diffusion and perfusion limited hypoxia. Omega three improve the tumor blood flow (microcirculation), acting simultaneously on these factors and on hemorheology.

Compared to the drugs mentioned, they have some peculiar actions that can be exploited together with radiotherapy, especially for prolonged high precision treatments such as low dose brachytherapy of brain primary with permanent implants. The first aspect is the direct cytotoxic activity on some tumor cell lines due to membrane lipoperoxidation (107). Moreover, lipoperoxidation deserves attention for its inverse dose rate effect during irradiation (50). For example, a significant radiation effect is obtained by membrane unsaturation at sufficiently low dose rates below 0.01 Gy (108). This greater response to radiotherapy and the increased mutagenic effects of certain products of lipid peroxidation, even at a low dose, may represent a possible way to generate neoplastic transformation in tumor neighbouring normal tissues. This, associated with the improvement in perfusion and consequently in oxygen content both in tumor and normal tissues, may augment the radiosensitivity of the latter and the severity of acute and late radiation-induced injury. This suspicion is probably incorrect. Studies by Horrobin seem to indicate a radioprotection in rats submitted to essential fatty acids and especially to GLA administration (16,17). w-3 share many common mechanisms of action with GLA, so it is possible to hypothesize a similar protective effect on normal tissues (60). A consideration of some importance is that not all the studies have confirmed an inhibitory effects of w-3 on tumor growth rate and that tumor cells differ in their sensitivity to EFAs; however, no studies have shown a mutagenic effect (63). The difference in sensitivity and the cycle phase arrest by EPA and DHA must be examined further; in fact, some authors have reported that DHA arrests the sensitive cells in G1 cycle phase, a radioresistant state (109). Burns and Spector have also

reported that tumor membrane unsaturation does not increase the radioresponse (59). This aspect must be verified again *in vivo*.

The second aspect is the capacity to reduce the expression of some oncogenes (c-*myc*, *ras*-P-21) and the activity of protein kinase C (94). This plays an important role during reoxygenation, a phenomena able to generate drug-radioresistance and metastasisation.

In our opinion, the major contribution of w-3 is the amelioration of blood flow - oxygen distribution inside the tumor mass and in microenvironment modification, suggesting further tests regarding their use in conjunction with radiotherapy.

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