Supplementation of the diet with eicosapentaenoic acid: a possible approach to the treatment of thrombosis and inflammation

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Metabolites of arachidonic acid (the general term ‘eicosanoids’ will be used in this article) have biological activities that suggest that they could influence some pathophysiological events. In this brief review only their role in thrombosis and inflammation will be considered. Another C20 fatty acid, eicosapentaenoic acid (EPA), is a poor substrate for some enzymes which efficiently transform arachidonic acid (AA). Some EPA metabolites have different quantitative and qualitative activities from those derived from AA. These findings have led to speculation that supplementation of the diet with EPA could be beneficial in the treatment or prevention of thrombosis and inflammation, or both. In the following sections the evidence for this hypothesis will be examined.

Role of AA metabolites in thrombosis

Two products of AA metabolism via the cyclo-oxygenase pathway are implicated in the control of haemostasis. Thromboxane A2 (TxA2), which is formed in blood platelets, induces aggregation of platelets and vasoconstriction. Prostacyclin (PGI2) counterbalances these effects since it is a vasodilator and inhibits platelet aggregation (see Bunting et al. 1983b). Both TxA2 and PGI2 are formed from the same precursors, the prostaglandin endoperoxides, PGG2 and PGH2. The presence of prostacyclin in vascular endothelium may explain why platelets do not adhere to or aggregate on healthy vasculature. It follows that any increase in the ratio, TxA2:PGI2, could lead to a thrombotic event. Decreased formation of PGI2 occurs in unhealthy vessels, e.g. in atheromatous plaques there is a high concentration of peroxides which are known to inhibit prostacyclin synthetase (Harland et al. 1973; Salmon et al. 1978; see also Dembinska-Kiec et al. 1977). There are a few reports of elevated levels of thromboxane in the blood of patients who have suffered a thrombotic episode, e.g. elevated levels of TxB2 (the stable hydrolysis product of TxA2) occur in Prinzmetal’s angina (Lewy et al. 1979). Also, plasma TxB2 is increased in shock induced in animals by endotoxin (Wise et al. 1980) and heterologous blood (Bunting et al. 1983a); both these animal models are characterized by platelet consumption.

Drug manipulation of TxA2:PGI2

The foregoing discussion suggests that inhibitors of TxA2 synthesis could be useful antithrombotic drugs. Aspirin, which inhibits the formation of prostaglandin endoperoxides from AA, is claimed to be antithrombotic, but results
from large and expensive trials have been disappointing. One explanation for the
poor clinical effect of aspirin is that, at normal doses, it blocks the formation of
PGI₂ as well as TxA₂. Thus, the potential antithrombotic benefits of PGI₂ are
eliminated. Consequently, attempts have been made to develop selective inhibitors
of thromboxane synthesis which have the potential of redirecting prostaglandin
endoperoxide metabolism to increased formation of PGI₂. However, even these
compounds (e.g. Dazoxiben; see Tyler, 1983) have provided insignificant clinical
benefit in thrombotic disorders (e.g. Rustin et al. 1983).

Dietary manipulation of TxA₂: PGI₂

EPA is a poor substrate for the fatty acid cyclo-oxygenase and therefore the
endoperoxides PGG₂ and PGH₁ are formed in low yield (Van Dorp, 1967;
Needleman et al. 1976). However, the endoperoxides themselves are efficiently
converted by thromboxane synthetase and prostacyclin synthetase to TxA₃ and
Δ¹⁷ prostacyclin respectively (Smith et al. 1979). It has been reported that whereas
Δ¹⁷ prostacyclin has an anti-aggregatory activity comparable to that of PGI₂ itself,
TxA₃ was not pro-aggregatory (Needleman et al. 1979; Gryglewski et al. 1979).
This led to speculation that supplementation of the diet with EPA could lead to a
reduced thrombotic tendency (Dyerberg et al. 1978). Indeed, epidemiological
studies support this concept: several communities who consume a high amount of
EPA in their diet, such as Greenland Eskimos and inhabitants of Japanese fishing
villages, have a reduced incidence of thrombo-embolic episodes compared with
age- and sex-matched control populations (Dyerberg & Bang, 1979; Hirai et al.
1980). Supplementation of a normal diet with fish, cod-liver oil or purified EPA
leads to decreased platelet aggregability and increased bleeding times (Seiss et al.
1980; Terano et al. 1983); these changes are considered to reflect an anti-
thrombotic effect.

Subsequent studies have indicated that TxA₃ is pro-aggregatory for platelets but
it is not as potent as TxA₂ and the aggregation is reversible (Gryglewski et al.
1979).

Although EPA is a poor substrate for the cyclo-oxygenase it does competitively
inhibit metabolism of AA and this is the most likely mechanism for the
antithrombotic properties of EPA: decreased synthesis of TxA₂ (measured as
TxB₂) has been observed following increased intake of EPA in the diet. In fact,
doubts were raised as to the formation of trienoic prostanoids in vivo after feeding
EPA-rich diets; however, recent studies in animals and humans have confirmed
that both TxA₃ and Δ¹⁷-prostacyclin can be formed (Hamberg, 1980; Dyerberg

Role of leukocytes in myocardial infarction

So far the discussion has centred on platelets being the major blood cell involved
in thrombotic events but the role of polymorphonuclear leukocytes (PMN) should
also be considered. In an animal model of myocardial infarction it was observed
that PMN accumulated in the area of infarction. The infarct was induced in
anaesthetized dogs by occluding the left anterior descending coronary artery for 1 h followed by reperfusion for periods up to 5 h (Mullane et al. 1984). Initially, the leukocytes adhered to the vascular endothelium and later it was observed that these cells had migrated into the sub-endothelium. It was suggested that the PMN contributed to the tissue damage in the infarct by releasing lysosomal enzymes and toxic oxygen radicals. Consequently, inhibition of PMN influx into the heart would reduce tissue damage. Indeed, hydroxyurea and an experimental compound, BW755C, both reduced PMN accumulation in the infarct and this correlated with a reduction in the area of the infarction (Mullane et al. 1984).

It is now known that AA is not only converted by the fatty acid cyclo-oxygenase to prostanoids but it also serves as a substrate for lipoxygenases. Some metabolites formed via the latter enzymes have leukotactic properties, i.e. they induce movement of leukocytes along the concentration gradient of the compound. In an early report, Turner et al. (1975) demonstrated that 12-hydroxy-eicosatetraenoic acid (12-HETE), which is formed from AA by the action of a 12'-lipoxygenase, is a leukotactic agent. In the model of myocardial infarction described previously, it was demonstrated that infarcted tissue synthesized significantly more 12-HETE than non-infarcted myocardium and this increase correlated with the infiltration of PMN (Mullane et al. 1984). It was established that 12-HETE was a major product of AA metabolism in dog PMN. Thus, although 12-HETE was probably not the initial signal for the influx of cells, it is possible that the synthesis of this leukotactic principle by the PMN in the infarcted tissue amplified the response.

Myocardial infarction has several similarities to acute inflammation: (1) release of chemical mediators, (2) cellular infiltration, (3) oedema, (4) pain and (5) loss of function. Therefore, although the following sections will concentrate on inflammation, the discussion is also relevant to myocardial infarction.

Formation and biological activities of leukotriene $B_4$

Although 12-HETE is an active leukotactic agent, another product of AA metabolism, leukotriene $B_4$ (LTB$_4$), is more potent in vitro and in vivo (see Bray, 1983). LTB$_4$ is formed from AA via the 5'-lipoxygenase in leukocytes; initially AA is converted to 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) which is successively transformed to an unstable epoxide, LTA$_4$, and then to LTB$_4$ (see Samuelsson, 1983). Since LTB$_4$ may be an important mediator of cell infiltration occurring in inflammation, the pharmaceutical industry is actively engaged in research designed to inhibit its synthesis; however, it is possible that this goal can be achieved by nutritional means.

Dietary manipulation of leukotriene synthesis

Although EPA is a poor substrate for the fatty acid cyclo-oxygenase, it is metabolized efficiently by lipoxygenases and pentaene leukotrienes can be formed (Nugteren, 1975; Jakshik et al. 1980). However, in several in vitro and in vivo tests, LTB$_5$ was considerably less active at affecting neutrophil function compared with LTB$_4$ (Terano et al. 1984a).
Synthesis of LTB₄ by leukocytes increased after giving rats a diet supplemented with EPA-ethyl ester (240 mg EPA/kg per d) for 4 weeks (Terano et al. 1984b). This feeding protocol also decreased the formation of LTB₄. There was a good correlation between the ratio, LTB₃:LTB₄ synthesis by leukocytes with EPA: AA in leukocyte phospholipids (Terano et al. 1984b).

If LTB₄ is an important mediator of cell infiltration occurring in inflammation, then the previously-mentioned findings suggest that EPA could be beneficial in the prevention or treatment of chronic inflammation, or both. The anti-inflammatory effects of EPA have been studied in two models of inflammation (Terano et al. 1985). First, the concentrations of PGE₂, TxB₂ and LTB₄ and the number of leukocytes in inflammatory exudates induced by implanting carrageenin-impregnated sponges subcutaneously were determined in rats given a normal diet and in rats provided with an EPA-rich diet (feeding regime described previously). The EPA-supplementation significantly reduced the synthesis of both PGE₂ and TxB₂; the concentration of LTB₄ and the number of leukocytes were also lowered but not significantly. Second, swelling was measured as an index of oedema formation after injection of carrageenin (20 g/l) into rat paws; supplementation of the diet with EPA significantly reduced the oedema relative to the control animals which is probably explained by lower synthesis of PGE₂ (Terano et al. 1985).

Thus, an EPA-enriched diet may reduce the severity of inflammatory reactions by decreasing the synthesis of dienoic prostanoids and tetraenoic leukotrienes. Also, EPA promotes the formation of LTB₄ which is considerably less biologically active than LTB₄ and may antagonize the pro-inflammatory action of LTB₄. However, this nutritional approach to anti-inflammatory therapy must be considered carefully because some AA metabolites may have anti-inflammatory effects. For example, PGE₂ has been shown to be an immunosuppressant and therefore reduction of its synthesis could produce a greater inflammatory response. Indeed, this is probably the mechanism by which an EPA-rich diet increased the incidence of collagen-induced arthritis in rats (Prickett et al. 1984). The anti-inflammatory benefits, or otherwise, of supplementing the diet with EPA will only be clarified by conducting well-controlled clinical trials. In general, an altered dietary intake of EPA can be expected to act in a long-term manner suited to preventative or prophylactic approaches rather than the short-term activity usually associated with pharmaceutical agents. Thus, experiments in animal models of human disease, which are necessarily compressed into a short time-scale, are probably inadequate for assessing the beneficial effect of EPA.

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