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 C_{20} 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 A_2 (TxA_2), which is formed in blood platelets, induces aggregation of platelets and vasoconstriction. Prostacyclin (PGI_2) counterbalances these effects since it is a vasodilator and inhibits platelet aggregation (see Bunting *et al.* 1983*b*). Both TxA_2 and PGI_2 are formed from the same precursors, the prostaglandin endoperoxides, PGG_2 and PGH_2. 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, TxA_2:PGI_2 could lead to a thrombotic event. Decreased formation of PGI_2 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 TxB_2 (the stable hydrolysis product of TxA_2) occur in Prinzmetal's angina (Lewy *et al.* 1979). Also, plasma TxB_2 is increased in shock induced in animals by endotoxin (Wise *et al.* 1980) and heterologous blood (Bunting *et al.* 1983*a*); both these animal models are characterized by platelet consumption.

Drug manipulation of TxA_2:PGI_2

The foregoing discussion suggests that inhibitors of TxA_2 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 PGI_2 as well as TxA_2. Thus, the potential antithrombotic benefits of PGI_2 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 PGI_2. 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 TxA_2:PGI_2

EPA is a poor substrate for the fatty acid cyclo-oxygenase and therefore the endoperoxides PGG_3 and PGH_3 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 TxA_3 and Δ^{17} prostacyclin respectively (Smith *et al.* 1979). It has been reported that whereas Δ^{17} prostacyclin has an anti-aggregatory activity comparable to that of PGI_2 itself, TxA_3 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 TxA_3 is pro-aggregatory for platelets but it is not as potent as TxA_2 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 TxA_2 (measured as TxB_2) 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 TxA_3 and Δ^{17}-prostacyclin can be formed (Hamberg, 1980; Dyerberg *et al.* 1981; Fischer & Weber, 1984).

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; Jakschik *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.* 1984*a*).

Synthesis of LTB_5 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.* 1984*b*). This feeding protocol also decreased the formation of LTB_5. There was a good correlation between the ratio, LTB_5: LTB_4 synthesis by leukocytes with EPA: AA in leukocyte phospholipids (Terano *et al.* 1984*b*).

If LTB_4 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 PGE_2, TxB_2 and LTB_4 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 PGE_2 and TxB_2; the concentration of LTB_4 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 PGE_2 (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 LTB_5 which is considerably less biologically active than LTB_4 and may antagonize the pro-inflammatory action of LTB_4. However, this nutritional approach to anti-inflammatory therapy must be considered carefully because some AA metabolites may have anti-inflammatory effects. For example, PGE_2 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.

REFERENCES

Bray, M. A. (1983). *British Medical Bulletin* **39**, 249–254.
Bunting, S., Castro, S., Salmon, J. A. & Moncada, S. (1983*a*). *Thrombosis Research* **30**, 609–617.
Bunting, S., Moncada, S. & Vane, J. R. (1983*b*). *British Medical Bulletin* **39**, 271–276.
Dembinska-Kiec, A., Gryglewska, T., Zmuda, A. & Gryglewski, R. J. (1977). *Prostaglandins* **14**, 1025–1034.
Dyerberg, J. & Bang, H. O. (1979). *Lancet* **ii**, 433–435.

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Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S. & Vane, J. R. (1978). *Lancet* **ii,** 117–119.
Dyerberg, J., Jorgensen, K. A. & Arnfred, T. (1981). *Prostaglandins* **22,** 857–862.
Fischer, S. & Weber, P. C. (1984). *Nature* **307,** 165–168.
Gryglewski, R. J., Salmon, J. A., Ubatuba, R. B., Weatherly, B. C., Moncada, S. & Vane, J. R. (1979). *Prostaglandins* **18,** 453–478.
Hamberg, M. (1980). *Biochimica et Biophysica Acta* **618,** 389–398.
Harland, W. A., Gilbert, J. D. & Brooks, C. J. W. (1973). *Biochimica et Biophysica Acta* **316,** 378–385.
Hirai, A., Hamazaki, T., Terano, T., Nishikawa, T., Tamura, Y., Kumagai, A. & Sajiki, J. (1980). *Lancet* **ii,** 1132.
Jakschik, B. A., Sams, A. R., Sprecher, H. & Needleman, P. (1980). *Prostaglandins* **20,** 401–410.
Lewy, R. I., Smith, J. B., Silver, M. J., Saia, J., Walinsky, P. & Wiener, L. (1979). *Prostaglandins and Medicine* **2,** 243–248.
Mullane, K. M., Read, N., Salmon, J. A. & Moncada, S. (1984). *Journal of Pharmacology and Experimental Therapeutics* **228,** 522–570.
Needleman, P., Minkes, M. & Raz, A. (1976). *Science* **193,** 163–165.
Needleman, P., Raz, A., Minkes, M., Ferrendelli, J. A. & Sprecher, H. (1979). *Proceedings of the National Academy of Sciences, USA* **76,** 944–948.
Nugteren, D. H. (1975). *Biochimica et Biophysica Acta* **380,** 299–307.
Prickett, J. D., Trentham, D. E. & Robinson, D. R. (1984). *Journal of Immunology* **132,** 725–729.
Rustin, M. H. A., Grimes, S. M., Kovacs, I. B., Cooke, E. D., Bowcock, S. A., Sowemimo-Coker, S. O., Turner, P. & Kirby, J. D. T. (1983). *European Journal of Clinical Pharmacology* **27,** 61–65.
Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S. & Vane, J. R. (1978). *Biochimica et Biophysica Acta* **523,** 250–262.
Samuelsson, B. (1983). *Science* **220,** 568–575.
Seiss, W., Roth, P., Scherer, B., Kurzmann, I., Bohlig, B. & Weber, P. C. (1980). *Lancet* **i,** 441–444.
Smith, D. R., Weatherly, B. C., Salmon, J. A., Ubatuba, F. B., Gryglewski, R. J. & Moncada, S. (1979). *Prostaglandins* **18,** 423–438.
Terano, T., Higgs, G. A., Salmon, J. A. & Moncada, S. (1985). *Biochemical Pharmacology* (In the Press).
Terano, T., Hirai, A., Hamazaki, T., Kobayashi, S., Fujita, T., Tamura, Y. & Kunagai, A. (1983). *Atherosclerosis* **46,** 321–331.
Terano, T., Salmon, J. A. & Moncada, S. (1984*a*). *Prostaglandins* **27,** 217–232.
Terano, T., Salmon, J. A. & Moncada, S. (1984*b*). *Biochemical Pharmacology* **33,** 3071–3076.
Turner, S. R., Tainer, J. A. & Lynn, W. S. (1975). *Nature* **257,** 680–681.
Tyler, H. M. (1983). *British Journal of Clinical Pharmacology* **15,** 13S–16S.
Van Dorp, D. A. (1967). *Progress in Biochemical Pharmacology* **3,** 71–82.
Wise, W. C., Cooke, J. A., Knapp, D. R. & Halushka, P. V. (1980). *Circulation Research* **46,** 854–859.

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