N-3 FATTY ACIDS, EICOSANOIDS AND CONTROL OF INFLAMMATION

by

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SUMMARY

This thesis addresses issues arising from the observation that dietary fish oils favourably modify experimentally-induced inflammation in animals and in human diseases including rheumatoid arthritis (RA). The anti-inflammatory effects described appear related to the presence of n-3 fatty acids in the fish oil, in particular eicosapentaenoic acid (EPA). EPA is a potential substrate for the enzymes 5-lipoxygenase (5-LO) and cyclooxygenase (CO) which are pivotal in the synthesis of lipid mediators of inflammation, known as eicosanoids. Although there have been many studies on the 5-LO metabolites of EPA, little is known about the the production or activity of the cyclooxygenase metabolite of EPA, prostaglandin E_3 (PGE_3). The studies undertaken for this thesis required the development of an assay for the measurement of PGE_3 and assessment of its biological activity. Possible interactions between conventional drug therapy and dietary fish oil supplements in therapeutic regimens designed to control inflammation were also investigated. Studies were undertaken to assess the mechanism for the putative inhibition of synthesis of leukotriene B_4 (LTB_4) by the anti-inflammatory agent methotrexate (MTX) since this drug is an effective anti-arthritic agent and the possibility of favourable drug/diet interactions was sought.

Adjuvant-induced arthritis in rats was used as a model of systemic inflammation and polyarthritis in which to investigate the effects of inflammation on the incorporation into leukocytes of dietary n-3 fatty acids. No effect on the rate or level of incorporation of EPA or depletion of arachidonic acid (AA) was seen and further studies were undertaken in normal animals.

The biological activity of PGE_3 with regard to oedema formation in mice was examined. Paw swelling was measured 30 minutes after injection of 10 μl PGE_2 or PGE_3 into the plantar region of the hind paw. Doses investigated ranged from 1 ng - 10 μg. Both PGE_2 and PGE_3 had substantial oedemogenic activity in this system.

An assay was developed which resolved PGE_3 from PGE_2. Prostaglandins E_1, E_2 and E_3 were derivatized with p-(9-anthroyloxy)phenacyl bromide (panacyl bromide)

and partly purified by thin layer chromatography (TLC). The PGs were further separated and analysed by reverse phase high pressure liquid chromatography (HPLC) with fluorometric detection. Human, rat and mouse adherent cells were incubated overnight and the culture medium extracted, derivatized and analysed for PG production. PGE_2 was detected in supernatants from cells from each species. PGE_2 synthesis was reduced following addition of EPA (5 μM) to the overnight culture. PGE_3 was not detected under these conditions. Studies were also undertaken using adherent cells from rats, mice and humans given dietary fish oil supplements rich in EPA. PGE_3 was not detected although the dietary intervention yielded substantial incorporation of EPA into cell membranes and LTB_5, a metabolite of EPA, was produced by leukocytes after appropriate stimulation and analysis by HPLC.

These observations suggest that although PGE_3 has inflammatory activity comparable to that of PGE_2, PGE_3 may not be generated in sufficient quantities to play a major role in mediating inflammatory reactions.

Studies were also undertaken to examine the effect of MTX *in vitro* and *ex vivo* on the production of the 5-lipoxygenase metabolites of arachidonic acid by rat and human neutrophils. MTX added *in vitro* to normal rat or human cells was weakly inhibitory and without a convincing dose response relationship. No inhibition of LTB_4 production by leukocytes was seen following administration of MTX orally to subjects with RA or by any of three routes of administration investigated in healthy rats (gavage, subcutaneous injection and intraperitoneal injection). The studies thus yield no support for earlier claims that MTX is an inhibitor of 5-LO and the possibility of an additive or synergistic effect of MTX and EPA on 5-LO metabolism was not pursued.