REGULATION OF MACROPHAGE FUNCTIONS BY POLYUNSATURATED FATTY ACIDS

ZHI HUA HUANG M.B.B.S., M.D.

Thesis submitted for the degree of Doctor of Philosophy

Department of Immunopathology

Women's and Children's Hospital

and

Department of Paediatrics

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SUMMARY

Recent studies have demonstrated that polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA, 20:4, *n-6*), eicosapentaenoic acid (EPA, 20:5, *n-3*) and docosahexaenoic acid (DHA, 22:6, *n-3*) can alter major cell responses in lymphocytes and neutrophils. To date, there is very little known on the effects of these fatty acids on macrophage function. Since a major reason for studying the interaction of PUFAs with leukocytes is to better define mechanisms of the inflammatory response and ways of applying PUFAs to treat infectious diseases and autoimmune/allergic diseases, it is important to study the effects of PUFAs on the mononuclear phagocytic system because of its role in the pathogenesis of these diseases.

The thesis focussed on investigations into the effects of PUFA on a key response of the mononuclear phagocyte, namely the respiratory burst involving NADPH oxidase activation. This was determined by the lucigenin-dependent chemiluminescence assay. A major finding was that the PUFAs, AA, EPA and DHA induced little or no production of superoxide in monocytes, macrophages and the monocytic cell line (HL-60) differentiated to macrophages. This is in direct contrast to the well-characterised substantial activation of the NADPH oxidase by these fatty acids in neutrophils. However, such PUFA treated

monocyte/macrophages showed significantly enhanced superoxide production in response to the tripeptide agonist, f-met-leu-phe (fMLP). These synergistic responses were characterized by a more rapid onset of the chemiluminescence response, as an increase in initial peak rate of chemiluminescence and longer duration of the response. It is interesting that, in relation to this synergistic response, the *n-3* PUFAs, EPA and DHA, were as active as AA (20:4, *n-6*) since *n-3* fatty acids have been implicated as anti-inflammatory fatty acids. The role of fatty acid structure in the ability to stimulate the fMLP response was also investigated. The data showed that a free carboxyl group was necessary and that the activity of the fatty acids decreased substantially as the number of double bonds decreased to two or fewer. The addition of a hydroxy- and hydroperoxy-group to the PUFA (products normally formed from the metabolism of PUFA via the lipoxygenase pathway) resulted in total loss of activity.

The mechanisms by which fatty acids induce their effects on mononuclear phagocytes were partially elucidated. The data showed that PUFAs not only enhanced the response of macrophages to a surface receptor acting agonists, fMLP, but also to agonists which act at post receptor levels, such as phorbol myristate acetate (PMA) which directly activates on protein kinase C (PKC) and Ca^{2+} ionophore which acts by increasing intracellular calcium concentration. In macrophages, PUFA stimulated the translocation of the PKC isozymes α, βI, βII and ε to a particulate fraction and the activity of extracellular signal-regulated protein kinase 1&2 (ERK1 and ERK2) of the mitogen-

activated protein kinase (MAP kinase) family. Using inhibitors of PKC and ERK we were able to establish that the priming of macrophages by PUFA occurs via PKC and ERK pathways. By pursuing transfection technology and introducing dominant negative mutants of signalling molecules as well as inhibitors of the upstream regulators of ERKs into macrophages, we were able to establish that ERK activation occurs via PKC, $p21^{ras}$ and raf-1 dependent mechanism.

These findings establish that PUFAs of the *n-6* and *n-3* types, while being poor activators of the NADPH oxidase, prime macrophages to become highly reactive to other agonists/mediators. This was observed as a synergistic superoxide production. The data suggest that this activity is restricted to certain structural elements of the fatty acid molecules. The mechanisms of the biological effects of the PUFAs in terms of intracellular signalling pathway were also partly defined.

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