Mechanism of action of polyunsaturated fatty acids in rheumatoid arthritis

J. WATSON, R. MADHOK, E. WIJELATH, H. A. CAPELL, J. GILLESPIE, J. SMITH and M. L. BYARS

*Biochemistry Division, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow G4 ONR, and †Department of Rheumatology, Glasgow Royal Infirmary, Glasgow G4 0SF, U.K.

There is now considerable evidence that inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor play a major role in chronic inflammatory diseases such as rheumatoid arthritis (RA) [1], the main site of which is the synovial lining of joints. Within the synovium, cells such as macrophages, fibroblasts and endothelial cells are capable of producing IL-1 and TNF [1] and it is therefore likely that these cytokines are involved in the initiation and prolongation of the inflammation of the synovium as well as directly causing cartilage erosion. RA is presently treated by a wide range of drugs, principally non-steroidal anti-inflammatory drugs or disease-modifying drugs (second line drugs), but a novel approach to the treatment of the disease has been suggested by several limited studies involving treatment of patients with polyunsaturated fatty acids [2]. These compounds appear to be beneficial in the treatment of disease and their effects could be due to a number of mechanisms including reduced production of pro-inflammatory cytokines, changes in cell membrane lipids altering transduction pathways and/or manipulation of eicosanoid products [3, 4].

In the present studies, monocyte cells were cultured with or without stimulation in the presence or absence of polyunsaturated fatty acids and both cellular proliferation and IL-1 secretion were measured. U937 cells are a monocytic tumour cell line which may serve as a model for human monocytes. The cells were grown at a density of 2 x 10⁴ cells/ml in 96-well microtitre plates (200 μl/well) in RPMI 1640 medium containing glutamine (2 mm), penicillin/streptomycin (100 i.u./ml) and foetal calf serum (v/v, 5%). The plates were incubated for 24 h at 37°C in a humidified incubator (5% CO₂/air) after which aliquots (4 μl) of a solution (2 mg of oil/ml) containing evening primrose oil or fish oil were added to appropriate wells. After a further 24 h incubation period under the same conditions, [³H]thymidine (0.5 μCi) was added to each well and the incubation continued for a further 4 h. Cells were harvested on to glass fibre discs using a Skatron Tierrett cell harvester and thymidine incorporation was determined by liquid scintillation spectrometry. IL-1 secretion by the cells was measured in cells cultured by the above technique but with the omission of [³H]thymidine. The IL-1 in the culture supernatants was measured by the D10.G4.1 cell bioassay techniques described by Hopkins & Humphreys [5]. In a limited preliminary experiment, monocytes were prepared from human volunteers and RA patients, and subjected to the same proliferation experiments with fish oil as described above for the U937 cells. The monocytes were separated from blood by Nycofenz Monocyte separation.

From Table 1 it can be seen that both evening primrose oil and fish oil, but not olive oil, cause a marked drop in cell proliferation which was not due to cell death as the viability of the cells was assessed by the Trypan Blue exclusion test. The reduction in cell proliferation was paralleled by a reduction in IL-1 secretion (Table 1) by both unstimulated and lipopolysaccharide (LPS)-stimulated cells. Indeed the levels of IL-1 in the unstimulated cells were generally lower in the presence of evening primrose or fish oil than in the control cells or olive-oil-treated cells. The LPS-stimulated secretion of IL-1 by the cells was markedly attenuated by fish oil, less so by evening primrose and to a slight extent by olive oil.

The results of the limited preliminary study with human monocytes demonstrated that in both RA patients and healthy individuals, fish oil is capable of reducing the amount of IL-1 produced by monocytes. In healthy individuals, low levels of IL-1 are markedly stimulated by LPS and this stimulation was considerably attenuated by fish oil. RA patients have high levels of IL-1 which are not affected by LPS but fish oil had the effect of inhibiting IL-1 secretion in both unstimulated and stimulated monocytes.

These results suggest that monocytes cultured in the presence of polyunsaturated fatty acids have their membrane phospholipids altered by the incorporation of the fatty acids which may then metabolize to form alternative cyclo-oxygenase and lipoxigenase products. Prostaglandins and leukotrienes have been shown to be involved in the regulation of IL-1 synthesis/release [4, 6] and the formation of less biologically active prostaglandins and leukotrienes may account for the effects of fish oil and evening primrose oil on monocyte IL-1 secretion. This work was supported by the Medical Research Council and Scotia Pharmaceuticals Ltd.

Table 1. Proliferation of U937 cells and IL-1 concentrations in the supernatants of the cells grown in the presence of oils high in polyunsaturated fatty acids

<table>
<thead>
<tr>
<th>Test material</th>
<th>Proliferation of U937 cells [% change from control ( = 100%)]</th>
<th>IL-1 concn. (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (medium only)</td>
<td>100</td>
<td>0.015</td>
</tr>
<tr>
<td>Control + LPS</td>
<td>83</td>
<td>0.23</td>
</tr>
<tr>
<td>Olive oil</td>
<td>83</td>
<td>0.011</td>
</tr>
<tr>
<td>Olive oil + LPS</td>
<td>38.3</td>
<td>0.041</td>
</tr>
<tr>
<td>Evening primrose oil</td>
<td>4.7</td>
<td>0.018</td>
</tr>
<tr>
<td>Evening primrose oil + LPS</td>
<td>4.7</td>
<td>0.018</td>
</tr>
<tr>
<td>Fish oil</td>
<td>4.7</td>
<td>0.018</td>
</tr>
<tr>
<td>Fish oil + LPS</td>
<td>4.7</td>
<td>0.018</td>
</tr>
</tbody>
</table>

References:

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The protective action of nitric oxide against membrane damage induced by myoglobin radicals

K. RICHARD BRUCKDORFER,* GARETH DEE,* M. JACOBS† and CATHERINE A. RICE-EVANS*

*Department of Biochemistry and Chemistry and †Department of Pharmacology, Royal Free Hospital School of Medicine, London NW3 2PF, U.K.

Myoglobin is able to transport molecular oxygen when the haem iron is in the Fe(II) oxidation state. In the cardiac myocyte some of the myoglobin is also in the iron (III) state of metmyoglobin. This can be oxidized further by H₂O₂ to give higher oxidation states including species with ferryl-like properties (Mb⁴⁺) and a transient free radical form of ferryl myoglobin (Mb⁴⁺v) is also formed [1, 2]. The radical forms of myoglobin may cause oxidative damage to essential cellular constituents, particularly cell membranes [3].

NO, a free radical, reacts with the haem of myoglobin and other haemproteins. NO is a potent vasodilator released from endothelial cells [4] and, under certain circumstances, may be released from macrophages. The aim of this work was to study the effects of NO on the formation and survival of ferryl species of myoglobin and the oxidative damage to membranes caused by these radicals.

Myoglobin (10–50 μM) in its various oxidation states shows characteristic visible spectra which were observed using a Beckman DU64 spectrophotometer with Quant 1 software to select specific wavelengths and measure absorbances (A). The proportions of the haem oxidation states were calculated according to the Whitburn equations as described in [5]:

\[
\text{Oxymyoglobin (II)} = 2.8 (A_{280}) - 127 (A_{410}) + 153 (A_{600})
\]

\[
\text{Metmyoglobin (III)} = 146 (A_{280}) - 108 (A_{410}) + 2.1 (A_{600})
\]

\[
\text{Ferryl myoglobin (IV)} = -62 (A_{280}) + 242 (A_{410}) - 123 (A_{600})
\]

The radical (Mb⁴⁺v) and non-radical (Mb⁴⁺) states of ferryl-myoglobin were not distinguishable by this technique.

Solutions of NO were prepared in helium-flushed water [4] and dispensed in gas-tight syringes. Haemoglobin-free erythrocyte membranes were prepared and the rate of formation of oxidation products resulting from the action of myoglobin radicals in the presence or absence of NO was determined spectrophotometrically as the amount of thiobarbituric acid-reactive substances/mg of membrane protein [3].

Oxymyoglobin is readily converted to metmyoglobin and ferryl-myoglobin in the presence of excess H₂O₂, whereas metmyoglobin is oxidized to the higher oxidation states ferryl-myoglobin (Mb⁴⁺v) and the myoglobin radical species (Mb⁴⁺v) [6]. When added to metmyoglobin or oxymyoglobin activated by H₂O₂, NO (30-fold excess over myoglobin concentration) induced a marked reduction in the concentration of ferryl species which were converted back to myoglobin (Fig. 1). However, the high concentration of H₂O₂ allowed reactivation of the myoglobin to the ferryl state after 5 min. When lower concentrations of H₂O₂ were used, the same concentrations of NO almost totally abolished the for-

![Fig. 1. Effects of NO (600 μM) on activated myoglobin induced by (a) 20 μM-metmyoglobin/400 μM-H₂O₂, (b) 20 μM-oxymyoglobin/400 μM-H₂O₂, (c) 20 μM-metmyoglobin/100 μM-H₂O₂, and (d) 20 μM-oxymyoglobin/100 μM-H₂O₂.](image-url)