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ANNOTATION

FREE RADICALS AND DUPUYTREN'S CONTRACTURE

Orthopaedic surgeons use free radical chemistry every time they implant a cemented prosthesis or make an incision. Indirectly free radicals play a part in inflammatory disorders and auto-immune diseases as well as in the action of some of the drugs used in their treatment. Recently they have also been shown to be important in the pathogenesis of Dupuytren's contracture. Clearly orthopaedic surgeons need to know about them.

What, then, are free radicals and what are their biological effects? Free radicals are simply compounds with unpaired electrons, that is, electrons each of which occupies an atomic or molecular orbit by itself. It is these chemically reactive compounds that are the intermediates in the polymerisation of methylmethacrylate monomer to the stable polymerised form. However, in biology the most important effects centre around the chemistry of oxygen free radicals.

One of the few biochemical facts we all remember is that aerobic oxidation is much more efficient than anaerobic oxidation or glycolysis; nearly ten times as much energy is produced from the aerobic oxidation of glucose to lactic acid. However, there is a price to pay. The price is that some of the oxygen consumed (about 1%) gives rise to the superoxide anion $(O_2)^-$ and other unstable oxygen species rather than stable water (H_2O) or carbon dioxide (CO_2).

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Human metabolism depends on the transfer of reducing equivalents from the organic components of the diet (fats, carbohydrates and proteins) to molecular oxygen. Molecular oxygen is itself a free radical, containing two unpaired electrons. This in part explains its reactivity, but in discussion of oxygen free radicals in biology it is the oxygen compounds with single free electrons that are the problem. Tissues contain many enzymes, such as peroxidases, catalases and superoxide dismutase, and compounds, such as glutathione and several antioxidants, which destroy these active oxygen species, emphasising the potential risks of unprotected aerobic metabolism.

Thus the reduction of oxygen during metabolism is never completely efficient and a series of unstable oxygen species is produced including the oxygen-derived free radicals (ODFR) O_2^- (superoxide) and OH (hydroxyl) (Joyce 1987). Apart from their formation during oxygen metabolism, these species may also be formed during prostaglandin synthesis and drug oxidation amongst other processes. The oxygen-derived free radicals are normally detoxified by specific enzyme systems together with many detoxicants. Vitamin A (carotene), Vitamin C (ascorbic acid), Vitamin E (tocopherol), glutathione and cysteine are all detoxicants present in vivo. They can mop up the ODFR by donating to them the needed single electrons.

Not all these oxygen free radicals are equally toxic. Thus superoxide does not attack proteins but in aqueous solutions it produces hydrogen peroxide. In effect

whenever superoxide is generated, hydrogen peroxide also is formed, and hydrogen peroxide, in contrast to superoxide, can enter cells.

Within cells hydrogen peroxide can form the hydroxyl radical, a reaction which is catalysed by free iron or copper ions. Usually tissue iron and copper are firmly bound by specific proteins but if for any reason these two free metal ions accumulate, hydroxyl radicals form from hydrogen peroxide. It is the generation of these hydroxyl free radicals that may account for many of the toxic effects of hydrogen peroxide. Thiol (–SH) compounds and Vitamin C will also form active free radical species in the presence of these two metal ions and molecular oxygen.

The hydroxyl radical is one of the most chemically reactive known. It can react with most components of living cells – proteins, DNA and the unsaturated fatty acids of membrane phospholipids. Specific degradation of proteins and DNA is catalysed by hydroxyl radicals. The hydroxyl radical is also perhaps the most active free radical in the peroxidation of unsaturated fatty acids (lipid peroxidation or the oxidative deterioration of polyunsaturated lipids). These latter reactions not only alter the properties of cell membranes but also control the overall flux of prostaglandin synthesis. The prostaglandins are themselves formed from unsaturated fatty acids by enzymic reactions catalysed by oxygen free radicals and are active mediators of many cellular signalling processes.

Excessive formation of free radicals is encountered during conditions of hyperoxia, in tissue ischaemia, radiation damage, during inflammation and with certain toxins (Dormandy 1983; Halliwell and Gutteridge 1985). These conditions then lead to a situation of oxidative stress (Wolff 1987), which can be defined as the exposure of biological systems to peroxide and oxygen free radicals in cells or tissues, beyond those usually encountered. The defence mechanisms mentioned above are overwhelmed, degradative changes in tissue lipids, protein and DNA result, and the clinical manifestations of oxygen – radical mediated disease follow.

In inflammation invading polymorphonuclear leucocytes and monocytes/macrophages are activated and elaborate large quantities of superoxide; this is the respiratory burst of white cells (Babior 1978). Normally this superoxide serves to lyse invading bacterial cells, but it may also mediate some of the tissue damage in autoimmune disease, such as the cartilage destruction in rheumatoid arthritis (Blake, Allen and Lunec 1987). Agents which suppress inflammatory activity such as the non-steroidal anti-inflammatory drugs and D-penicillamine may be able to scavenge oxygen radicals in vitro but whether this property contributes to their actions in vivo is uncertain.

Heritable defects in superoxide production by leucocytes occur and cause chronic granulomatous disease. Heritable defects also occur in the cellular defence mechanisms used against oxygen derived free radicals, as in glucose-6-phosphate dehydrogenase deficiency which leads indirectly to lowered levels of reduced glutathione, an important detoxicant of free radicals.

Oxygen-derived free radicals are formed during the metabolism of many drugs and toxins (Mason and Chignell 1981). Many carcinogens can form free radicals, as can antineoplastic agents (Editorial, Lancet 1985). Radiation damage also occurs by stimulating oxygen radical generation and damage, and oxygen radical detoxicants, such as dimethylsulphoxide, reduce the toxicity of radiation experimentally (Biaglow 1982). Radio-protective drugs will need to have these properties.

Oxygen-derived free radicals may mediate acute ischaemic damage. Under conditions of hypoxia (and also hyperoxia) the enzyme xanthine oxidase is formed from xanthine dehydrogenase. The altered enzyme catalyses the formation of superoxide from oxygen mediated oxidation of hypoxanthine and xanthine (produced from ATP in situations of relative hypoxia). This process may contribute to tissue necrosis around regions of myocardial and cerebral infarction and of the gut as in the necrotising enterocolitis of infants (Parks, Bulkley and Granger 1983; Dalsing et al. 1983).

Recent evidence has been presented that there is a greater potential for superoxide generation in Dupuytren's contracture. Production of free radicals may be an important feature of the pathogenesis of Dupuytren's contracture and other fibrotic conditions (Murrell, Francis and Bromley 1987). These workers have shown that in Dupuytren's contracture the abnormal fibrotic fascia contains abnormal concentrations of hypoxathine which, on reacting with the xanthine oxidase of the vessel walls, releases oxygen free radicals. These then damage the perivascular connective cells and this damage is repaired by fibrosis.

Free radical production need not necessarily be deleterious. The use of a burst of free radical production by white cells in their lysis of bacteria has already been mentioned. Low levels of superoxide and other free radicals may stimulate fibroblast proliferation (Murrell et al. 1987) and this could be important in normal wound healing. Peroxide levels (tone) within cells may modulate intracellular calcium ion levels and prostaglandin metabolism as well as controlling the relative metabolic activity within different sub-cellular structures such as the mitochondria and the cytosol.

Knowledge of oxygen radical formation and of the physiological mechanisms that prevent tissue damage from these chemically destructive molecules increases. Relationships between excess oxygen radical production and clinical disease are being documented, but methods of controlling oxygen radical production clinically are still limited. Perhaps the possible success of allopurinol (an inhibitor of xanthine oxidase) in the treatment of Dupuytren's contracture (Murrel et al. 1987) and of D-

penicillamine in rheumatoid arthritis are forerunners of further therapeutic strategies, for treating ischaemic damage, neoplastic and radiation injury and also fibrotic conditions.

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