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**Iron free radicals and arthritis**

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HYPOTHESIS

In the present review we propose that the mobile and inflamed human joint is subject to cycles of ischaemia followed by reperfusion. Ischaemic reperfusion cycles lead to iron decompartmentalization which promotes oxygen radical damage. Reactive O<sub>2</sub> species have the capacity to cause damage to a variety of biomolecules in the joint, eventually leading to a persistent and locally destructive inflammatory process. Fe overload appears to selectively worsen joint inflammation whilst nutritional Fe deficiency has the converse effect. We speculate that nutritional Fe deficiency suppresses joint inflammation by reducing the activity of the enzyme xanthine oxidase (*EC* 1.1.3.22).

THE JOINT: ITS BLOOD SUPPLY

The normal human body contains 187 synovial joints, in which the whole of the joint cavity, with the exception of the cartilage, is lined by synovium. The synovium is a thin sheet of modified vascular connective tissue of mesenchymal origin, which lacks basement membrane. The cavity of the joint, which can be considered to be a tear in the mesenchymal tissue, is an interstitial space occupied by fluid through which nutrients flow to reach the articular cartilage. Supplying nutrients, and carrying away waste products, there is a rich capillary network within the synovium and also in the underlying tissues. The synovium is well vascularized, considerably more so than the structures supporting the joint such as the capsule, ligaments and tendons (Henderson & Edwards, 1987). Castor (1960) found that 10% of all cells within 70 µm of the surface of the human knee synovium were endothelial cells. The majority of the cells were found within 25 µm of the synovial intimal surface. Goldie (1970) studying inflamed synovium noted that in patients with rheumatoid arthritis there was oedema and multiple erythrocyte extravasation from dilated capillaries and venules. There was tortuosity of vessels associated

with a decrease in linear flow velocity, but no thrombosis. Similar changes, though of a lesser degree, were found in osteoarthritis, indicating a similarity of the basic pathological process of inflammation in different types of synovitis, irrespective of the initiating stimulus.

#### THE JOINT: INTRA-ARTICULAR PRESSURE

In any compartmental system with a limited capacity for volume expansion, increasing the volume of fluid within it will lead to a rise in pressure within that compartment. This principle, we believe, applies to the joint. Joints that are swollen (increased volume) and tense (increased pressure) are a common clinical entity. The swelling may be due to soft tissue hypertrophy or to increased synovial fluid. An increase in the fluid volume has been demonstrated to raise the resting intra-articular pressure (Jayson & Dixon, 1970a). The synovial fluid volume of the normal human knee joint is 0.5–4 ml (McCarty, 1979), with an associated intra-articular pressure which is sub-atmospheric (approximately –2 mm Hg) (Reeves, 1966). In inflammatory joint disease the resting intra-articular pressure is raised, resting levels ranging from 6 to 33 mm Hg (Caughy & Bywaters, 1963). Normal exercise does not raise the intra-articular pressure in the normal joint; however, excessive exercise (20 min cycling on an exercise bicycle using 5 kg friction) will raise the intra-articular pressure in the normal knee to approximately 5 mm Hg, considerably below the capillary perfusion pressure (Reeves, 1966). In contrast, in inflamed joints with effusions, exercise considerably raises the intra-articular pressure (Jayson & Dixon, 1970b; Blake *et al.* 1989). In chronic inflammatory states, the simple exercise of tensing the quadriceps will increase intra-articular pressure to levels ranging from 112 to 300 mm Hg (well above the capillary perfusion pressure) whilst levels of 24–144 mm Hg are achieved simply by weight bearing (Caughy & Bywaters, 1963).

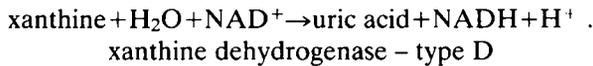
#### THE RELATIONSHIP BETWEEN INTRA-ARTICULAR PRESSURE AND SYNOVIAL BLOOD SUPPLY

Given that the pressure in an inflamed joint can rise above the systolic blood pressure on movement, it follows that the synovial capillary blood supply may be impaired when inflamed joints are moved. Using the novel technique of laser Doppler flowmetry to measure blood flow directly within the synovium, we have found that the synovial blood supply is indeed suppressed when the inflamed joint with an effusion is exercised (Blake *et al.* 1989). Geborek *et al.* (1989) have subsequently confirmed our observations and demonstrated that an increase in intra-articular pressure of as little as 20 mm Hg may significantly decrease synovial blood flow. We have found such pressure rises are not present in patients with acute traumatic effusions, almost certainly due to reflex muscle inhibition (Merry *et al.* 1989b). In the knee this is due to inhibition of a monosynaptic spinal reflex, the Hoffman reflex, that controls quadriceps tone (Spencer *et al.* 1984).

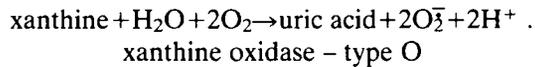
#### HYPOXIC REPERFUSION INJURY

Clearly depriving a tissue of O<sub>2</sub> will produce injury. The O<sub>2</sub> tension both within the effused synovial cavity and within the inflamed synovium is low (Falchuk *et al.* 1970). Low O<sub>2</sub> concentrations halt mitochondrial oxidative phosphorylation and cellular ATP

production becomes dependent on anaerobic glycolysis. This is an inefficient means of ATP production from glucose resulting in the production of lactic acid (Jennings & Reimer, 1981; Jennings *et al.* 1981). Synovial lactic acid levels are raised in inflammatory joint disease and have a positive correlation with the extent of inflammation (Falchuk *et al.* 1970). Increasing levels of lactic acid, together with an increasing NADH:NAD ratio eventually lead to the inhibition of glycolysis; moreover, the already reduced intracellular ATP and ADP levels fall further. This leads to raised levels of adenosine and its breakdown products, including hypoxanthine and xanthine, which are substrates for the enzyme xanthine dehydrogenase (EC 1.1.1.204; Jennings *et al.* 1981). The enzyme xanthine dehydrogenase is a molybdenum-containing flavoprotein with Fe-sulphur clusters. It oxidizes hypoxanthine and xanthine to uric acid. The enzyme is widely distributed in tissues and is present in normal and inflamed synovium. The enzyme reduces  $\text{NAD}^+$  as follows (Della Corte & Stirpe, 1972):



When tissues are homogenized without precaution, there is *in vitro* conversion of the dehydrogenase form to an oxidase form as a result of sulphhydryl oxidation or limited proteolysis. The oxidase form is unable to reduce  $\text{NAD}^+$ , but uses molecular  $\text{O}_2$  as an electron acceptor instead, as follows:



The conversion of the D form to the O form has been shown to occur *in vivo* in ischaemic tissues (Roy & McCord, 1983). During ischaemia the fall in cell energy leads to a failure of ion equilibrium across the cell membrane. As a consequence, the calcium ion concentration in the cytosol increases and appears to activate a protease capable of converting xanthine dehydrogenase to xanthine oxidase (McCord, 1985). This conversion has been demonstrated to occur within synovial tissue (Allen *et al.* 1987). On reperfusion the O form of the enzyme supplies  $\text{O}_2$  as an electron acceptor and high levels of hypoxanthine produce a flux of  $\text{O}_2^-$ . In addition, the  $\text{O}_2^-$  formed from the O form of the enzyme is capable of mobilizing Fe from the intracellular storage protein ferritin (Biemond *et al.* 1986). Such Fe will then have the capacity to form the highly toxic oxidizing species – the hydroxyl radical ( $\text{OH}^\cdot$ ) from  $\text{O}_2^-$  and hydrogen peroxide via the Fenton reaction (Halliwell & Gutteridge, 1985).

#### HYPOXIC REPERFUSION INJURY AND OXIDATIVE DAMAGE WITHIN THE SYNOVIAL CAVITY

We have previously demonstrated that inflammatory synovial fluid contains Fe in a form capable of generating the hydroxyl radical (Blake *et al.* 1984). This we believe is derived from Fe within the synovial membrane, which has diffused into the synovial cavity as a consequence of chronic hypoxia and subsequent reperfusion damage. We were interested to establish whether oxidative damage would occur to critical biomolecules as a consequence of joint exercise-induced hypoxic-reperfusion injury. Lipid peroxidation is commonly used as an index of radical-mediated reperfusion injury because polyunsaturated fatty acids are prone to attack by reactive  $\text{O}_2$  species. Rearrangements of a

methylene-interrupted double bond after hydrogen abstraction initiates a chain reaction involving the formation of lipid peroxide intermediates and ultimately fragmentation products and aldehydes which react with thiobarbituric acid (TBA; Aust & Svingen, 1982). In a blinded controlled study of thirty-four patients with inflammatory joint disease, we found that exercise significantly increased the amount of TBA-reactive material (Blake *et al.* 1989). Similar results were found utilizing the novel assay system based on second-derivative electronic absorption spectroscopy. In this more selective assay system, we found an increase in *trans*, *trans*- and *cis*, *trans*-conjugated diene lipid hydroperoxide as well as an increase in malonaldehyde derived from the breakdown of these hydroperoxides (Merry *et al.* 1989a).

Proteins, as well as lipids, are known to be susceptible to oxidative damage. Immunoglobulin (IgG) and its sub-classes have a high reactivity with reactive O<sub>2</sub> species, and the Fc and hinge regions which contain antigenic sites for rheumatoid factor are particularly susceptible to oxidation. Lunec *et al.* (1985) have shown that human IgG exhibits altered fluorescence and sulphhydryl damage when exposed to reactive O<sub>2</sub> species. In a study of nineteen patients with inflammatory joint disease, we have demonstrated a considerable rise in the ratio of fluorescent IgG to total IgG following a brief exercise regime (Blake *et al.* 1989).

From these studies we concluded that the mobile inflamed human joint is indeed subject to recurrent transient ischaemic episodes which lead to oxidative damage to both lipid and protein. Given the critical nature of polyunsaturated fatty acids as an integral part of cell membranes and the tendency of oxidatively damaged protein to form immune complexes, it is clear how the simple act of movement will initiate self-perpetuating inflammatory cascades.

#### THE ROLE OF IRON

The inflamed synovium is subject to recurrent traumatic microbleeding (Muirden & Senator, 1968). Early animal studies performed by Key (1929) showed that even a single injection of autologous blood in the rabbit knee led to a proliferation of synovial lining cells, and transient infiltration of both leukocytes and macrophages. In an inflamed environment we have demonstrated that the addition of haem-Fe in the form of autologous blood both amplifies and prolongs the inflammatory reaction (Yoshino *et al.* 1985; Morris *et al.* 1987). Animal models of systemic Fe overload are also associated with joint inflammation. Injection of either iron dextran or iron sorbitol to rats with adjuvant arthritis, at the onset of joint inflammation, leads to a significant exacerbation of joint inflammation along with Fe deposition in the synovium. In addition, iron sorbitol produced extensive focal osteoporosis when compared to either saline (9 g sodium chloride/l) or sorbitol-citrate complex controls (Dabbagh *et al.* 1988). In recent studies using a model of hydrogen peroxide-initiated inflammation in the rat knee joint, intravenous iron dextran significantly exacerbated joint inflammation (Dabbagh *et al.* 1989). In early rheumatoid patients the amount of synovial membrane ferritin has been significantly associated with the activity of the disease at the time of the biopsy, and the amount of Perls' (ferric-Fe) is associated with persistence of the disease (Blake *et al.* 1984). In detailed ultrastructural studies of a variety of inflammatory arthritides, Fe deposits found within siderosomes were mainly observed in the B cells of the rheumatoid synovium (Morris *et al.* 1986). Despite the obvious toxic effects of Fe on joint tissue,

certain rheumatoid patients require Fe therapy for genuine Fe-deficiency anaemia. Both total dose iron dextran (Blake *et al.* 1985; Winyard *et al.* 1987) and oral Fe (Blake & Bacon, 1982) have been shown to exacerbate inflammatory arthritis. In patients treated with iron dextran the exacerbation of synovitis occurs when the transferrin is saturated and Fe with the capacity to cause oxidative damage (low-molecular-weight Fe complexes) is present within synovial fluid. It is of interest, in view of our understanding of hypoxic-reperfusion injury in the joint, that a mobile joint exhibits far greater Fe-mediated inflammatory exacerbation than a rested joint. It is also of interest that there are no reports of iron dextran promoting non-infective inflammation at other sites other than the joint in man.

This apparent selective effect of Fe on joint inflammation was also noted in studies of Fe deficiency on models of inflammation in the rat. We have shown that nutritional Fe deficiencies produced by feeding rats with a diet containing 10 mg Fe/kg leads to a mild anaemia and decreased liver Fe stores. On such a diet, the chronic symmetrical arthritis associated with adjuvant disease in rats is suppressed, though no effect was noted on the more systemic components of the disease (acute phase response, liver pathology, and lymph node hyperplasia) (Andrews *et al.* 1987). Further studies showed that the same level of Fe deficiency had no effect on the development of an acute inflammatory response to carageenan, pyrophosphate or uric acid crystals in models of inflammation not involving the joint. Likewise the same level of Fe deficiency did not cause a depression of cell-mediated immunity in response to oxazolone.

#### SPECULATION CONCERNING THE SELECTIVE EFFECT OF IRON IN JOINT INFLAMMATION

Fe may act in a variety of ways on the inflammatory cascade (Halliwell & Gutteridge, 1986). For example, nutritional Fe deficiency has been associated with a suppression of immune function. Fe-deficient patients have a decreased percentage of T lymphocytes and an impaired incorporation of [<sup>3</sup>H]thymidine by stimulated lymphocytes in culture, and a depressed delayed hypersensitivity response. Nutritionally Fe-deficient rats have been reported to have impaired humoral as well as cell-mediated immunity. If, in our adjuvant disease model, nutritional Fe deficiency had been acting in either of these ways, we would not have anticipated such a selective effect on joint inflammation and, indeed, at the modest level of Fe deficiency in our experiments, we observed no effect on oxazolone hypersensitivity nor any effect on thymidine incorporation by lymphocytes. Fe catalysed oxidative radical injury within the inflamed mobile joint, promoting reperfusion injury could, however, explain the peculiarly selective effects observed. As stated previously, the enzyme xanthine oxidase appears to have a central role in reperfusion injury, and contains a number of Fe-S centres. Nutritional Fe deficiency has been shown to depress xanthine oxidase activity within the rat (Kelley & Amy, 1984). It is tempting to speculate that a similar effect occurs within the inflamed synovium.

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