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## *Leaders*

# Inhibition of xanthine oxidase by allopurinol: A therapeutic option for ischaemia induced pathological processes?

Allopurinol was synthesised in the late 1950s to inhibit the oxidative degradation of mercaptopurine (for review see Ref 1). By that time there seemed to be no feasible way of increasing the rate of mercaptopurine conversion to thioinosinic acid, its cytotoxic nucleotide derivative. Further research focused on possible inhibition of the mercaptopurine major degradative pathway, which involves oxidation by xanthine oxidase to the inert compound thiouric acid. It was soon recognised that allopurinol could reduce the effective dose of mercaptopurine severalfold. In addition, allopurinol prevented increased serum urate concentrations and urinary uric acid excretion produced by chemotherapy, and the compound has since been almost routinely used for this purpose.[2–4] Inhibition of xanthine oxidase by allopurinol is particularly useful in diseases due to or complicated by hyperuricaemia, hyperuricosuria, or urinary urate stone formation,[5] with an adverse reaction rate of about 3·5%.[6] In recent years a growing number of experimental studies have indicated that oxygen derived free radicals may be involved in the pathogenesis of diverse disease states, including chronic inflammatory polyarthritis. In this leader we examine the role of xanthine oxidase catalysed reactions in reperfusion tissue damage and summarise data already published suggesting that allopurinol may be beneficial in ischaemia mediating pathological processes.

### Link between xanthine oxidase and reperfusion injury

Enhanced capillary permeability and oedema formation are subtle indicators of ischaemic injury. When ischaemia is maintained, leakage of cytosolic enzymes causes more pronounced damage, which may be manifested by microscopic or gross macroscopic changes, and will ultimately produce tissue death. A substantial body of evidence indicates that oxygen derived free radicals play a major part in producing the microvascular and parenchymal damage associated with reperfusion of ischaemic tissues. McCord *et al* suggested that one of the most active sources for increased intracellular generation of oxygen free radicals in tissues after ischaemia is the enzyme xanthine oxidase,[7 8] particularly in those organs characterised by high enzyme activities, such as the small intestine and liver.[9] This proposal is based on three lines of evidence: (*a*) xanthine oxidase is a well documented biological source of oxygen radicals[10]; (*b*) xanthine oxidase is present in a wide variety of tissues[9]; and (*c*) allopurinol provides protection against diverse tissue injuries associated with ischaemia-reperfusion.[8] This hypothesis implies the conversion of xanthine dehydrogenase to xanthine oxidase during ischaemia coupled with the availability of molecular oxygen and purine substrates, hypoxanthine and xanthine, according to the following sequence (Fig. 1): When a tissue becomes ischaemic a number of pathological events occur, including depletion of cellular stores of high energy adenine nucleotides (ATP, ADP, AMP). This leads to a build up of hypoxanthine and xanthine, which serve as oxidisable purine substrates.[11–13] In addition, the cell's energy charge reduction limits the maintenance of an adequate ion gradient across its membranes. The resulting increased cytosolic calcium concentration has been proposed to activate a protease[7] which converts xanthine dehydrogenase, the originally synthesised form of xanthine oxidase that accounts for about 90% of the total activity in a healthy tissue and is $NAD^+$ dependent, to xanthine oxidase which is oxygen dependent.[14] When reperfusion takes place oxygen availability is restored to a tissue (*a*) with high concentrations of oxidisable substrates (hypoxanthine and xanthine) and (*b*) with an enzyme that uses molecular oxygen (xanthine oxidase). After addition of xanthine to xanthine oxidase the enzyme generates superoxide radicals ($\cdot O_2^-$), which can react with superoxide dismutase

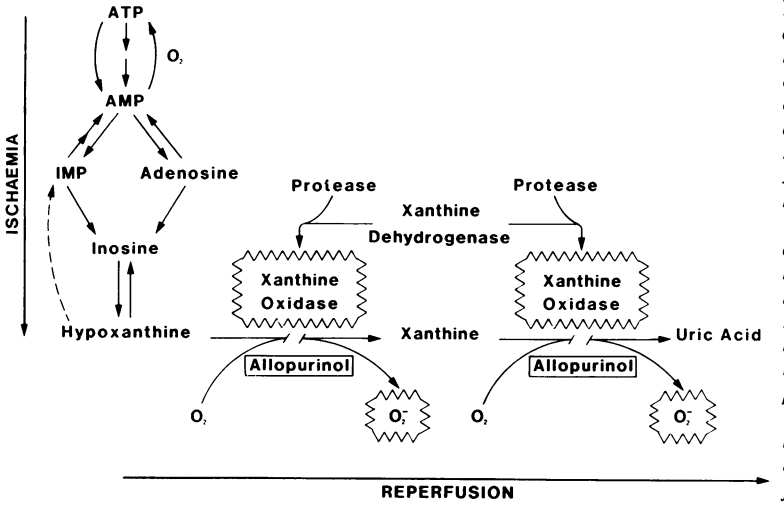


Fig. 1 *Proposed mechanisms of allopurinol protection against ischaemia-reperfusion tissue damage. Allopurinol is a scavenger of hydroxyl radicals (·OH), which are formed from superoxide radicals ($\cdot O_2^-$). Inhibition of xanthine oxidase by allopurinol may limit superoxide generation. This enzymatic inhibition results in an increased availability of hypoxanthine for purine nucleotide synthesis (dashed line). In addition, allopurinol conversion to allopurinol ribonucleotide inhibits 5'-nucleotidase and prevents inosine monophosphate (IMP) and adenosine monophosphate (AMP) dephosphorylation, thereby facilitating adenosine triphosphate (ATP) synthesis.*

to form hydrogen peroxide ($H_2O_2$) in proportions dictated by the pH, and superoxide and xanthine concentrations.[15] Superoxide and hydrogen peroxide can further generate the powerful oxidant hydroxyl radical (·OH), depending upon the presence of suitable transition metal catalysts, such as iron or copper.[16] Iron metabolism has been implicated in the pathogenesis of rheumatoid arthritis. Increased concentrations of iron salts have been found in the synovial fluid of patients with rheumatoid arthritis,[17] and ferritin correlates with indices of inflammatory activity.[18 19]

Reactive oxygen species are capable of reversibly or irreversibly damaging compounds of all biochemical classes, including nucleic acids, proteins and free amino acids, lipids and lipoproteins, carbohydrates, and connective tissue macromolecules.[20 21] In addition to the xanthine oxidase system, other possible sources of oxygen radical production at the moment of reperfusion include the electron transport chain,[22] leucocytes,[23] and certain oxidative enzymes such as cytochrome P-450 reductase.[24] Oxygen free radicals have been implicated in ischaemia-reperfusion injury in multiple tissues, including the brain,[25] heart,[26–28] stomach,[29] small intestine,[30 31] pancreas,[32–34] kidney,[35] liver,[36–39] muscle,[40] and skin flaps.[41] Evidence for this implication comes from studies in which the administration of a superoxide generating system (a purine base with xanthine oxidase and oxygen) caused tissue damage,[42–45] and from controlled animal experiments in which radical scavengers attenuated tissue injury following ischaemia-reperfusion.[26 30 32 42 46–48] Recently, several authors measured directly in vitro free radical concentrations by electron paramagnetic resonance.[49–52] This technique is similar to nuclear magnetic resonance in that a sample is placed within a magnetic field and energy is absorbed by a paramagnetic particle. The spectra obtained allow distinct identification of the individual free radical species generated. During the period of ischaemia little change in radical composition was observed.[49] When reperfusion started, however, a burst of free radical production[49] or stable products reacting with these radicals[50–52] were measured. Thereafter radical production decreased rapidly with time.[49] Direct evidence of free radical production in the intact animal was recently provided by Bolli *et al.*[53] By means of a spin trap these authors showed that the intensity of oxygen radical generation by the reperfused myocardium was related to the severity of ischaemia and was maximal two to four minutes after restoration of blood flow (90 times above ischaemic values). Thereafter the release of the trapped radical declined but continued for up to three hours after reperfusion. These studies provide direct evidence to support the hypothesis that reactive oxygen species are mainly generated within a few minutes after restoration of blood flow.[49–53] This concept is particularly important in delineating possible therapeutic interventions as, to be effective, a free radical scavenger would have to be present at the moment of reperfusion; any brief delay would probably reduce the efficacy of the agent.

The above pathogenic mechanism may be applicable to the inflamed synovium. In fact several clinical observations have suggested that chronic

synovial inflammation can also be due to hypoxic-reperfusion injury mediated by oxygen radicals.[54] Blake *et al* recently reported,[55] in inflammatory synovitis due to several rheumatic conditions, that exercise of the knee is associated with (*a*) an increase in intra-articular pressure that exceeds the capillary perfusion pressure, (*b*) a fall in synovial fluid $Po_2$ followed by an increase to suprabasal levels after exercise, (*c*) a mean reduction of the synovial capillary perfusion of 90% from baseline values, and (*d*) a significant rise in lipid peroxidation products and in fluorescent IgG, which indicates free radical damage to the protein.[56] Free radical modification of IgG may render this molecule antigenic and reactive with rheumatoid factor, thereby promoting immune complexes formation and chronic inflammation.[57] These results strongly support the hypothesis that chronic synovial inflammation may be mediated by reactive oxygen species generated during exercise induced hypoxia-reperfusion. In an attempt to define both the cellular source and the oxygen radical species produced Zweier *et al* showed that endothelial cells subjected to anoxia-reoxygenation generated superoxide derived hydroxyl radicals, which caused cell damage, and that most of the oxygen radical production was derived from the xanthine oxidase enzyme system.[58] This system is present in both normal and diseased synovial tissue.[59] On the other hand, normal human synovial fluid contains no superoxide dismutase, catalase, or glutathione peroxidase to protect against the potential damage produced by oxygen free radicals.[60]

**Possible strategies to prevent oxygen free radical reperfusion injury**

From a theoretical point of view two main strategies could be proposed to prevent free radical mediated reperfusion injury: reduction of oxygen radical formation and administration of free radical scavenging agents. Some authors believe that, among the various potential sources of free radicals, xanthine oxidase mediated degradation of purine bases is the most important source, and thus is a potential target for therapeutic intervention.[7 8 54 61 62] In fact the administration of allopurinol has been shown to protect against haemorrhagic shock induced gastric lesions,[63] to prevent increased vascular permeability associated with intestinal[64] and skeletal muscle ischaemia,[40] to ameliorate experimental acute pancreatitis,[33] to protect renal[65] and liver[39] function after ischaemia, to reduce myocardial infarct size elicited by coronary artery ligation[66–69] and reperfusion induced arrhythmias,[12 68] and to improve the survival rate of skin flaps,[70] and the survival of dog kidney[71 72] and liver[73] after transplant.

The mechanism by which allopurinol exerts a protective effect in ischaemia-reperfusion injury is probably multifactorial (Fig. 1). Allopurinol and oxypurinol are scavengers of the highly reactive hydroxyl radicals.[62] Allopurinol and oxypurinol may also limit superoxide generation through xanthine oxidase inhibition.[7 8] In addition, allopurinol and oxypurinol may be converted to their corresponding ribonucleotides by the enzyme hypoxanthine phosphoribosyltransferase.[74] Allopurinol ribonucleotide causes a 50% inhibition of 5′-nucleotidase at a concentration of 10 μmol/l.[75] This may prevent the dephosphorylation of inosine and adenosine monophosphates, thereby facilitating ATP resynthesis. Furthermore, inhibition of xanthine oxidase by allopurinol elicits an increased availability of hypoxanthine that may be salvaged for purine nucleotide synthesis.[76 77] Enhancement of hypoxanthine reutilisation may save a substantial quantity of energy[78] as five molecules of ATP are required for inosine monophosphate synthesis via *de novo* pathway, whereas the conversion of hypoxanthine to inosine monophosphate uses only one molecule of ATP for phosphoribosylpyrophosphate synthesis. This energy sparing pathway may be crucial for adequate organ function after a transplant. ATP recovery after global or regional ischaemia via *de novo* purine synthesis usually requires one to seven days,[79–81] and it has been shown that the ability of rat liver to regenerate its ATP and to maintain an adequate energy charge during restoration of hepatic blood flow determines tissue viability and the survival of the animal.[82] In addition, loss of adenine nucleotides appears to be a good marker of human liver graft damage,[83] and total adenine nucleotide concentration during cold storage has been related to the viability of the graft.[83] Preliminary experiments in our laboratory have shown that the decrease in dog liver ATP after 30 minutes of partial warm ischaemia was from (mean (SD)) 1·97 (0·23) to 1·10 (0·54) μmol/g of wet tissue ($p<0·001$). In contrast, when allopurinol (50 mg/kg of body weight) was infused for 30 minutes before and during ischaemia the decrease was from 2·22 (0·15) to 1·96 (0·22) μmol/g of wet tissue ($p>0·05$). The mean decrease in total adenine nucleotides (ATP plus ADP plus AMP) in control dogs at the end of the ischaemic period was −0·82 μmol/g of wet weight, whereas in dogs pretreated with allopurinol total adenine nucleotides remained essentially unchanged (Mateos FA, Puig JG, Delgado VD, manuscript in preparation). Restoration of adenine nucleotides in the reperfused rat[84] or dog[85] liver after ischaemia was not in-

fluenced by allopurinol pretreatment. Our results are similar to those reported in ischaemic kidneys[86] and emphasise the ability of allopurinol to prevent total adenine nucleotide depletion during ischaemia. In other ischaemic settings, however, allopurinol promoted total adenine nucleotide repletion,[87] probably as a consequence of enhanced hypoxanthine reutilisation.

Inhibition of xanthine oxidase by allopurinol may not be a universal mechanism by which this drug protects ischaemic tissues. Some studies using the rabbit heart, which is devoid of measurable xanthine oxidase activity, showed that allopurinol improves postischaemic left ventricular function[88] and preserves the myocardial ATP content.[89] This led to the postulation that additional protective mechanisms of allopurinol, in addition to its oxygen radical scavenging effect,[62] may include an enhanced antioxidant capacity of myocardial tissue.[89]

## Future directions

An increasing body of knowledge has implicated free radical mediated processes in a wide spectrum of different types of human diseases. Among the various potential strategies for reducing oxygen free radical toxicity there is significant evidence that allopurinol exerts a protective effect through several related but not completely understood mechanisms. We are aware of no studies specifically designed to evaluate whether free radical scavenger agents, such as allopurinol, could be beneficial in clinical situations associated with severe tissue damage after significant ischaemia and reperfusion. As we look back on the advances in the treatment of uric acid related diseases we can only hope that similar progress will be made in the elucidation of the intricacies of free radical formation and their interaction with other systems.[90] Further studies in this area of basic and integrated clinical research should provide fascinating insights into physiological and pathological processes, and would ultimately dictate relevant advances in protecting the body against free radical mediated diseases, such as myocardial ischaemia, inflammatory diseases, or rheumatic conditions.

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