Free radicals and ...

Oxidants and free radicals in inflammatory bowel disease

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The tissue injury and dysfunction associated with ulcerative colitis and Crohn's disease may be promoted by soluble mediators released from the phagocytic leucocytes that accumulate within the intestinal and colonic interstitium during active disease.[1] To engulf and destroy invading microorganisms, neutrophils, eosinophils, monocytes, and macrophages synthesise and release copious amounts of toxic reactive oxygen metabolites (ROMs).[2] Because ROMs are produced during normal metabolism and because this production may be increased dramatically during inflammation, cells and tissues have developed an extensive array of protective enzymic and non-enzymic antioxidants that will decompose these potentially injurious oxidising agents. During the inflammatory response these defences degrade most oxidants that escape phagocytic cells, thereby limiting injury to the surrounding tissue until the inflammatory response is down-regulated. However, sustained production of ROMs, as during chronic inflammation, would overwhelm the defences and damage the tissue oxidatively. This pro-oxidative imbalance created by the overproduction of ROMs has been termed "oxidative stress".

The chronically inflamed intestine and/or colon is subjected to significant oxidative stress.[3,4] Whether phagocytic leucocytes initiate or exacerbate gut injury and dysfunction is unclear. However, pharmacological or immunological inhibition of phagocyte recruitment, function, and/or mediator release attenuates some of the intestinal injury and dysfunction associated with experimental and human inflammatory bowel disease (IBD).[3,4]

Activity of leucocyte-derived oxidants

Interaction of pro-inflammatory agents, such as leukotriene B_4, platelet-activating factor, immune complexes, complement components, or bacterial products, with specific receptors on the phagocyte's plasma membrane activates the plasma-membrane-associated NADPH oxidase.[2] The result is the production and release of large amounts of superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) as well as oxidants derived via myeloperoxidase, such as hypochlorous acid (HOCl), and N-chloramines (RNHCl; figure). In addition to their cytotoxic properties, hydrogen peroxide, hypochlorous acid, and certain N-chloramines have other actions (table 1), which include increasing resting tension of ileal smooth-muscle strips while inhibiting the contractility of such strips after electrical stimulation.[4] Furthermore, H_2O_2 and certain RNHCls promote mutagenesis in prokaryotic and eukaryotic systems.[4,5]

In addition to the classic superoxide-driven Fenton reaction, oxidants with reactivity similar to the hydroxyl radical may be generated by the interaction between hydrogen peroxide and haemoproteins such as haemoglobin or myoglobin to yield activated haem plus aminoacid radicals, both of which can oxidatively damage lipids, protein, and carbohydrates.[6] Another mechanism by which reactive radicals may be generated at sites of inflammation is by the interaction between superoxide and the free-radical nitric oxide ($NO^{\bullet}$) to produce peroxynitrite.[7] Active episodes of colonic inflammation in humans or animal models of IBD are associated with enhanced nitric oxide production.[8,9] Nitric oxide or metabolites derived from it may have an important role in mediating some of the lesions of experimental IBD.[9] For example, nitric oxide rapidly and spontaneously reacts with molecular oxygen to yield a variety of nitrogen oxides:

$$2NO^{\bullet} + O_2 \longrightarrow 2NO_2$$
$$2NO^{\bullet} + 2NO_2 \longrightarrow 2N_2O_3$$
$$2N_2O_3 + 2H_2O \longrightarrow 4NO_2^- + 4H^+$$

where NO_2, N_2O_3, and NO_2^- represent nitrogen dioxide, dinitrogen trioxide, and nitrite, respectively. Dinitrogen trioxide and nitrogen dioxide are potent oxidising and N-nitrosating agents. Nitric oxide or species derived from it mediate cellular injury and may enhance electrolyte and water secretion.[10] Whether or not nitric oxide mediates its pro-inflammatory activity directly or indirectly via its

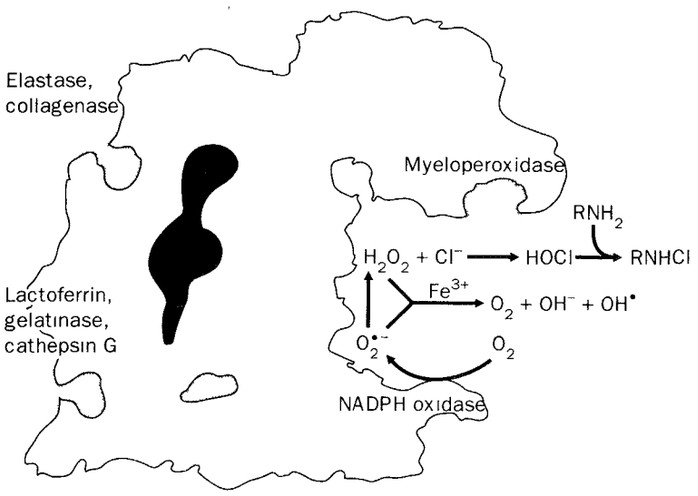

Figure: **Reactive oxygen metabolism of activated phagocytic leucocytes**

Activation of NADPH oxidase (see text) releases large amounts of superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) which can interact in presence of trace amounts of iron (Fe) to yield the hydroxyl radical ($OH^{\bullet}$). Hydroxyl radical or an oxidant with similar reactivity may also be formed by interaction between superoxide and hypochlorous acid (HOCl) and superoxide and nitric oxide. Activated phagocytes also secrete myeloperoxidase into extracellular space where it catalyses formation of hypochlorous acid with subsequent formation of N-chloramines (RNHCl).

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Physiological response	Oxidant
Enhanced chloride secretion	H_2O_2, HOCl, RNHCl, NO•
Alterations in smooth-muscle contractility	H_2O_2, HOCl, RNHCl
Mutagenic activity	H_2O_2 (OH•), RNHCl, N_2O_3
Enhanced mucosal permeability	H_2O_2, HOCl, RNHCl

OH• = hydroxyl radical.

Table 1: **Physiological activities of reactive metabolites of oxygen and nitrogen**

interaction with oxygen or superoxide remains to be determined.

In addition to direct effects, neutrophil-derived oxidants may damage the epithelium and mucosal interstitium indirectly by altering the protease/anti-protease balance in intestinal interstitium. For example, hypochlorous acid (and possible other lipophilic N-chloramines and reactive nitrogen intermediates) may inactivate protease inhibitors, such as α_1-protease inhibitor and α_2-macroglobulin, in the extracellular fluid to allow uncontrolled proteolysis by elastase.[11] The extracellular myeloperoxidase system (hypochlorous acid) may activate the latent collagenase and gelatinase secreted by neutrophils.[11] The data suggest that oxidative inactivation of important protease inhibitors coupled with the oxidant-mediated activation of latent proteases creates an environment favourable for elastase, collagenase, and gelatinase mediated degradation of the mucosal interstitial matrix and epithelial cells.

Overproduction of ROMs in chronically inflamed gut

Keshavarzian et al[12] and Simmonds et al[13] have demonstrated with chemiluminescence that the inflamed colons from human beings or from animals with experimental colitis produce much larger amounts of reactive oxygen species than control or uninvolved colons. In addition, Oshitani et al,[14] using the histochemical localisation of superoxide production via the reduction of nitroblue tetrazolium, reported that vascular endothelial cells and invading monocytes in patients with ulcerative colitis produce greater amounts of superoxide than those in control gut.

The reactivity of the more potent ROMs produced by phagocytic leucocytes dictates that the overproduction of ROMs within the inflamed mucosa and/or submucosa should result in the oxidative modification of various biological substrates, thereby providing "footprints" of oxidative stress. Indeed Ahnfelt-Ronne et al[16] found that colonic biopsy specimens from patients with active IBD had enhanced levels of lipid peroxidation products. These findings suggest that chronic gut inflammation promotes an imbalance between pro-oxidant and antioxidant mechanisms leading to the net accumulation of oxidatively modified proteins and lipids.

ROMs in chronic gut inflammation: cause or consequence?

Tissue-associated antioxidants can be overwhelmed during active gut inflammation, resulting in oxidative modification of cellular components. This is not surprising because human colonic mucosa, submucosa, and muscularis/serosa contain much smaller amounts of superoxide dismutase, catalase, and glutathione peroxidase than does liver.[16,17] Most of the mucosal enzyme activity is associated with colonic epithelial cells, so the lamina propria is devoid of significant enzymic defences against ROMs. The imbalance created by the overproduction of ROM within the inflamed interstitium suggests that antioxidant supplementation may prove useful in the treatment of IBD.

In an uncontrolled phase II trial, Emerit et al[18] reported that intramuscular injections of bovine copper/zinc superoxide dismutase attenuated inflammation and mucosal injury in 26 patients with severe Crohn's disease. Keshavarzian et al[19,20] demonstrated that intraperitoneal injections of superoxide dismutase (bound to polyethylene glycol), catalase, or non-specific antioxidants (eg; WR-2721, Cu-DIPS) modestly but significantly lessen the injury and inflammation produced by intra-rectal acetic acid, as judged by semiquantitive histological inspection.

ROMs have been implicated as mediators of gut inflammation because of the beneficial effects of mesalazine (5-aminosalicylate), the active metabolite of oral sulphasalazine. Although sulphasalazine has been used for over 40 years, the mechanism by which mesalazine exerts its anti-inflammatory activity in vivo remains speculative; possibilities include inhibition of cyclooxygenase and/or lipoxygenase activities, and of mitogen-stimulated secretion of immunoglobulins from mononuclear leucocytes (table 2).[21] However, the concentrations of mesalazine required to inhibit these reactions range from 1 to 10 mmol/L, which is substantially higher than the 0·1–0·2 mmol/L range achieved experimentally in the normal colonic mucosal interstitium.[22] Although active inflammation might increase mucosal permeability and thus the interstitial concentration of drug, we have found that permeability in a model of very severe colitis (eg, acetic-acid colitis) increases only 5-fold and not by the 10–100 fold that would be required to produce interstitial concentrations of mesalazine necessary to inhibit the above pathways. Although certain inhibitors of 5-lipoxygenase or 5-lipoxygenase activating protein suppress leukotriene B_4 synthesis in vivo, they are barely effective or inactive in clinical studies. This observation suggests that inhibition of leukotriene synthesis is not an important pathway by which mesalazine attenuates gut inflammation. An alternative mechanism involves mesalazine's potent antioxidant and free-radical-scavenger properties. This aminosalicylate decomposes superoxide and scavenges various oxygen, nitrogen, and haemoprotein-associated free radicals as well as non-radical oxidants (table 2). Furthermore, mesalazine inhibits the iron-catalysed, hydroxyl-radical-mediated degradation of deoxyribose by chelating iron and rendering it poorly redox active.[21]

	IC_{50} (μmol/L)
Cyclooxygenase inhibitor	10 000
Lipoxygenase inhibitor	6000
Inhibitor of neutrophil function	
Phagocytosis	NE
Chemotaxis	NE
Degranulation	NE
FMLP binding	>5000
Inhibitor of antibody secretion	1050–1350
Antioxidant	
Superoxide radical scavenger	10–20
Hydroxyl radical scavenger	400–1000
Carbon, peroxyl, or nitrogen centred free-radical scavenger	5–10
Hypochlorous acid scavenger	25–400
Iron chelator	300
Scavenger of haemoprotein-associated oxidants	20–50

*IC_{50} = that concentration necessary to inhibit the various reactions by 50%. NE = no effect, FMLP = n-formyl-methionyl-leucyl phenylalanine.

Table 2: **Proposed mechanisms of action of mesalazine**

A direct role for ROMs in chronic gut inflammation remains to be defined. A lack of long-lived antioxidants with well-characterised mechanisms of action and of specific inhibitors of phagocyte-associated reactive-oxygen generators hampers research. We await the development of new-generation antioxidants and inhibitors.

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