Leading article

Oxygen radicals: mediators of gastrointestinal pathophysiology

Recent evidence suggests that reactive oxygen metabolites are mediators of the microvascular and parenchymal cell injury associated with ischaemia and subsequent reperfusion. Under normal conditions, approximately 95% of molecular oxygen in biological systems undergoes controlled reduction through the addition of four electrons (tetravalent) in the mitochondrial cytochrome oxidase system to form water.¹ The remaining molecular oxygen undergoes sequential, univalent reduction to produce the partially reduced oxygen free radical intermediates, superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH). Nature has provided a myriad of antioxidant enzymes and scavengers to protect against the deleterious effects of these highly reactive metabolites of oxygen. The primary defence against oxidative insults to the tissue include the enzymes, superoxide dismutase, catalase, and glutathione peroxidase. Superoxide dismutases are a family of predominantly intracellular metallo-proteins that catalyse dismutation of O_2^- to H_2O_2 . Only trace amounts of superoxide dismutase activity are detected in extracellular fluid – that is, plasma, spinal fluid and lymph.² Two enzyme systems are important in regulating the intracellular concentration of H_2O_2 . Catalase is a haemo-protein which catalyses decomposition of H_2O_2 to water. Glutathione peroxidase is a cytoplasmic, selenium containing enzyme which catalyses decomposition of H_2O_2 or organic peroxides to water or the corresponding organic alcohol. The antioxidant activity of glutathione peroxidase is coupled to the oxidation of reduced glutathione to oxidised glutathione which can be subsequently reduced by glutathione reductase using NADPH as the reducing agent. A selenium independent form of glutathione peroxidase has recently been described.¹ In addition to antioxidant enzymes, a multitude of water soluble – for example, ascorbic acid, cysteine, and lipid soluble – for example, α -tocopherol, β -carotene, antioxidants provide a second line of defence against oxidant induced tissue injury.

Sources of oxygen radicals

Oxygen free radicals may be formed from several sources, including: components of the mitochondrial electron transport system; endoplasmic reticulum; prostaglandin synthetase and lipoxygenase systems; soluble enzymes and proteins; and autoxidation of several compounds and xenobiotics. Of the multitude of potential sources of reactive oxygen metabolites, two major enzymes have been implicated in the tissue damage associated with ischaemia reperfusion, xanthine oxidase, and neutrophilic NADPH oxidase.

XANTHINE OXIDASE

Xanthine oxidase is the most well documented biological source of reactive oxygen species and has been detected in a large number of bacteria, with representatives from at least eight phyla and innumerable classes of the animal kingdom. Of all the mammalian tissues studied, liver and small intestine contain the greatest xanthine oxidase activity.³ Although xanthine oxidase activity in tissue homogenates has been well quantified, the cellular localisation of xanthine oxidase remain uncertain. Immunofluorescence microscopy indicates that xanthine oxidase activity is limited to the capillary endothelium.⁴ This contrasts with the histochemical evidence suggesting that the cytosol of intestinal and hepatic epithelium contains considerable xanthine oxidase activity.⁵ An epithelial localisation of xanthine oxidase is also suggested by the observation that isolated hepatocytes metabolise hypoxanthine to uric acid and allantoin in a process inhibited by allopurinol, a competitive inhibitor of xanthine oxidase.⁶ Finally, considerable xanthine oxidase activity has been detected in freshly isolated intestinal epithelium and hepatocytes (Parks, unpublished results).

In normal, non-ischaemic tissues, xanthine oxidoreductase exists predominantly as an innocuous, NAD⁺-reducing dehydrogenase which can be converted to an oxygen radical producing oxidase (xanthine oxidase) through oxidation of essential sulphhydryl groups (reversible) or by limited proteolysis (irreversible). The rate of xanthine dehydrogenase to xanthine oxidase conversion in the rat small intestine is the most rapid of all tissues in the gastrointestinal tract, where nearly 95% conversion occurs in less than one minute ischaemia.⁷ The resulting oxidase could not be reconverted to xanthine dehydrogenase with sulphhydryl reducing agents, consistent with proteolytic conversion. This contention is supported by the observation that serine protease inhibitors attenuate the increased vascular permeability associated with intestinal ischaemia.⁸ Parks *et al*⁹ suggests a much slower rate of xanthine dehydrogenase to xanthine oxidase conversion in ischaemic rat intestine than originally proposed. In the liver, conversion of xanthine dehydrogenase to xanthine oxidase is considerably slower than in intestine.¹⁰ Parks *et al*¹¹ have shown that ischaemia causes release of massive amounts of xanthine oxidase into the perfusate of isolated perfused rat liver concomitant with release of other hepatocellular enzymes. In addition, xanthine oxidase is markedly raised in the circulation of rats subjected to haemorrhagic shock. As xanthine oxidase activity in the perfusate far exceeds the activity required to damage isolated endothelial cells,¹² it has been postulated that this circulating xanthine oxidase would be capable of extensive damage to vascular endothelium and responsible for multiple tissue dysfunction. This hypothesis is supported by evidence that xanthine dehydrogenase+xanthine oxidase is raised to 50 times normal in the serum of patients with infectious hepatitis and in some patients with extrahepatic jaundice.¹³

NEUTROPHILS

The plasma membrane associated NADPH oxidase of neutrophils, monocytes, eosinophils and macrophages is another potential source of reactive oxygen metabolites. The NADPH oxidase reduces molecular oxygen to O₂⁻ with the concomitant oxidation of cytosolic NADPH. Unlike the xanthine oxidase system which generates approximately 20% O₂⁻ and 80% H₂O₂

under normoxic conditions at physiologic pH, the NADPH oxidase generates predominately O_2^- with H_2O_2 as a secondary product through spontaneous dismutation of O_2^- .¹ Neutrophils also contain high concentrations of myeloperoxidase, which can use the H_2O_2 generated by NADPH oxidase to oxidize halides.

Oxygen radicals in ischaemia reperfusion injury

SMALL INTESTINE

The present evidence suggests that reactive oxygen metabolites are responsible for the parenchymal and microvascular changes associated with intestinal ischaemia reperfusion. Pretreatment with superoxide dismutase markedly attenuates the increased vascular permeability¹⁴ and morphological alterations¹⁵ associated with ischaemia reperfusion. The observation that reduced glutathione is consumed with a concomitant formation of oxidised glutathione in the intestinal mucosa after ischaemia reperfusion¹⁶ is also consistent with oxidant mediated tissue injury. This suggestion is supported by the observation that lipid peroxidation products are doubled after reperfusion of the ischaemic intestine¹⁶ and that the increase is largely prevented by superoxide dismutase.

The enzyme xanthine oxidase is probably a major source of reactive species of oxygen formed during reperfusion of the ischaemic small intestine. The strongest support for involvement of xanthine oxidase in ischaemia induced tissue injury is the observation that allopurinol and oxypurinol, competitive inhibitors of xanthine oxidase, are as effective as superoxide dismutase and catalase in reducing the increased vascular permeability¹⁴ and morphologic alterations^{15,17} associated with ischaemia reperfusion. Pterin aldehyde, structurally dissimilar from allopurinol, also attenuates the increase in microvascular permeability associated with ischaemia reperfusion.¹⁸ Inactivation of xanthine oxidase with a tungstate rich, molybdenum poor diet attenuates the increased vascular permeability associated with reperfusion of ischaemic intestine and strongly implicates xanthine oxidase as a primary source of the oxidants.¹⁹ Infusion of approximately 2 mU/ml xanthine oxidase into the arterial supply of non ischaemic small intestine results in increased microvascular permeability similar to that observed after one hour ischaemia reperfusion.²⁰ This increased microvascular permeability was largely prevented by superoxide dismutase or dimethyl sulphoxide (DMSO), a putative OH scavenger. Additional support for involvement of xanthine oxidase in ischaemia induced tissue injury is derived from the observation that the reperfusion induced consumption of reduced glutathione (and concomitant formation of oxidised glutathione) and increased indicators of lipid peroxidation is attenuated by administration of allopurinol.¹⁶ These observations suggest that xanthine oxidase derived oxidants are responsible for the tissue injury associated with reperfusion of ischaemic small intestine.

Another potential source of oxygen radicals in ischaemic small intestine is neutrophilic NADPH oxidase. Grisham *et al*²¹ reported that granulocyte infiltration was increased on reperfusion of ischaemic intestine. Both superoxide dismutase and allopurinol largely prevented the granulocyte infiltration. These results were extended by the observation that other antioxidants (catalase, deferoxamine or dimethylthiourea (DMTU)) atten-

uated the infiltration of granulocytes.²² Further support for involvement of neutrophils in intestinal injury is provided by the observation that depletion of circulating neutrophils with antisera or prevention of adherence to endothelium by monoclonal antibodies²³ provides significant protection against reperfusion induced injury. These data suggest that xanthine oxidase derived oxidants attract and activate granulocytes which exacerbate ischaemia induced tissue injury.

STOMACH

Oxygen radicals have been implicated in the gastric mucosal injury associated with ischaemia reperfusion. Administration of superoxide dismutase reduced reperfusion induced red blood cell loss across the cat²⁴ and rat²⁵ gastric mucosa and attenuated the formation of gross lesions in rats exposed to haemorrhagic shock.²⁶ Extracellularly generated oxidants damage isolated rat gastric mucosal cells as determined by release of ⁵¹Cr from prelabelled cells.²⁷ The primary mediator of the mucosal injury appears to be H₂O₂ with tissue injury attenuated by catalase and exacerbated by depletion of intracellular reduced glutathione which detoxifies H₂O₂. Xanthine oxidase appears to be a primary source of the reactive oxygen metabolites based on the observation that allopurinol attenuates post-ischaemic increased RBC clearance in the isolated cat stomach²⁴ and gross lesion formation in the rat stomach.²⁶ Additional evidence suggesting xanthine oxidase as a primary source of the oxidants is based on the observation that use of a tungstate diet to inactivate xanthine oxidase reduced red blood cell clearance and gross lesion formation after haemorrhagic shock.²⁵

GASTROINTESTINAL MUCUS

The surface epithelium of the gastrointestinal tract is covered by a continuous layer of insoluble mucus gel adherent to the epithelium. The gastrointestinal mucosa is constantly exposed^{28, 29} to luminal oxidants from ingested food – for example, a mixture of iron salts and ascorbic acid, frequently consumed in multiple vitamin preparations forms OH, catalase negative bacteria (produce large quantities of H₂O₂), oxidases from desquamated cells (xanthine oxidase and other oxidases) and saliva (hypothiocyanous acid formed from interaction of salivary peroxidases with H₂O₂ and thiocyanate). Because certain sugars are potent OH scavengers and mucin contains very high concentrations of similar sugars, it is hypothesised that gastrointestinal mucin may have physiologically important antioxidant properties.²⁸ Certainly mucin effectively scavenges OH generated from the iron-catalysed decomposition of H₂O₂.²⁹

LIVER

Oxygen radicals probably mediate some of the structural and functional alterations associated with reperfusion of ischaemic liver. Administration of superoxide dismutase and catalase attenuate the release of enzymatic indicators of hepatocellular injury^{30, 31} and improve the function of hepatic grafts³¹ of liver exposed to ischaemia. Treatment with the antioxidants, α -tocopherol³² or coenzyme Q₁₀³³ increase the survival, accelerate resynthesis of ATP, lower the consumption of water soluble (reduced glutathione) and lipid soluble (α -tocopherol) antioxidants and suppress the rise in lipid

peroxide associated with hepatic ischaemia reperfusion. The concentration of hepatic reduced glutathione decreases progressively during ischaemia³³ with a corresponding increase in oxidised glutathione and is attenuated by formate, a cell permeable OH scavenger.³⁴ Administration of reduced glutathione before induction of ischaemia significantly accelerates the resynthesis of ATP and reduces the formation of lipid peroxides upon reperfusion.³³ Conversely, a reduction in hepatic reduced glutathione, induced pharmacologically or by fasting, results in more extensive hepatocellular injury after ischaemia reperfusion than observed in liver with normal reduced glutathione content.³⁵ Several reports in the literature suggest that xanthine oxidase is a major source of the reactive oxygen species produced during reperfusion of ischaemic liver based on the observation that allopurinol is as effective as oxygen radical scavengers in attenuating the injury associated with hepatic ischaemia.^{30,31} The role of neutrophils in ischaemia reperfusion injury to the liver remains undefined but clearly warrants further attention.

Conclusions

Reactive oxygen metabolites are probably responsible for the tissue injury observed in several tissues of the gastrointestinal tract after ischaemia reperfusion. The formation of oxygen radicals from xanthine oxidase is considered a primary mechanism of tissue injury associated with ischaemia reperfusion. There is additional evidence that neutrophils play a major role in ischaemia induced injury. Considerable progress has also been made in defining the role of oxygen radicals in ischaemic injury using indirect approaches such as administration of antioxidants. Existing data would suggest therapeutic efficacy of antioxidant enzymes. Further studies are required that utilise more direct approaches for detection of free radical intermediates (electron spin resonance, chemiluminescence). Difficulties in assessing ischaemic injury also indicate a need for more detailed studies of drug specificity and the determination of the relative importance of the sources of oxidants.

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References

- 1 Weiss S. Oxygen, ischemia and inflammation. *Acta Physiol Scand* 1986; **126** (suppl 548): 9–38.
- 2 Marklund SL. Extracellular superoxide dismutase in human tissues and human cell lines. *J Clin Invest* 1984; **74**: 1398–403.
- 3 Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol Scand* 1986; **126** (suppl 548): 87–100.
- 4 Jarasch ED, Bruder G, Heid HW. Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol Scand* 1986; **126** (suppl 548): 39–46.
- 5 Auscher C, Amory N, Pasquier C. Localization of xanthine oxidase activity in hepatic tissue. A new histochemical method. *Adv Exp Med Biol* 1977; **76**: 605–9.
- 6 Vincent MF, Van Den Berghe G, Hers HG. Metabolism of hypoxanthine in isolated hepatocytes. *Biochem J* 1983; **222**: 145–55.
- 7 Roy RS, McCord JM. Superoxide and ischemia: Conversion of xanthine dehydrogenase to xanthine oxidase. In: Greenwald RA, Cohen G, eds. *Oxygen radical and their scavenger systems*. Volume II. *Cellular and medical aspects*. New York: Elsevier Biomedical, 1983: 145–53.

- 8 Parks DA, Granger DN, Bulkley GB, Shah AK. Soybean trypsin inhibitor attenuates ischemic injury to the feline small intestine. *Gastroenterology* 1985; **89**: 6-12.
- 9 Parks DA, Williams TK, Beckman JS. Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a re-evaluation. *Am J Physiol* 1988; **254**: G768-74.
- 10 McKelvey TG, Hollwarth ME, Granger DN, Engerson TD, Landler U, Jones HP. Mechanisms of conversion of xanthine dehydrogenase to xanthine oxidase in ischemic rat liver and kidney. *Am J Physiol* 1988; **254**: G753-60.
- 11 Yokoyama Y, Parks DA. Circulating xanthine oxidase: release of xanthine oxidase from isolated rat liver [Abstract]. *Gastroenterology* 1988; **94**: A607.
- 12 Freeman BA, Crapo JD. Biology of disease: Free radicals and tissue injury. *Lab Invest* 1982; **47**: 412-26.
- 13 McHale A, Grimes H, Coughlan M. Human serum xanthine oxidase: fluorometric assay applicable to the investigation of liver disorders. *Int J Biochem* 1979; **10**: 317-9.
- 14 Granger DN, Rutilli G, McCord JM. Superoxide radicals in feline intestinal ischemia. *Gastroenterology* 1981; **81**: 22-9.
- 15 Parks DA, Bulkley GB, Granger DN, Hamilton SR, McCord JM. Ischemic injury to the cat small intestine: Role of superoxide radicals. *Gastroenterology* 1982; **82**: 9-15.
- 16 Younes M, Mohr A, Schoenberg MH, Schildberg FW. Inhibition of lipid peroxidation by superoxide dismutase following regional intestinal ischemia and reperfusion. *Res Exp Med* 1987; **187**: 9-17.
- 17 Morris JB, Haglund U, Bulkley GB. The protection from postischemic injury by xanthine oxidase inhibition: blockade of free radical generation or purine salvage. *Gastroenterology* 1987; **92**: 1542-6.
- 18 Granger DN, McCord JM, Parks DA. Xanthine oxidase inhibitors attenuate ischemia-induced vascular permeability changes in the cat intestine. *Gastroenterology* 1986; **90**: 80-4.
- 19 Parks DA, Henson JL, Granger DN. Effect of xanthine oxidase inactivation on ischemic injury to the small intestine. *The Physiologist* 1986; **29**: 101.
- 20 Parks DA, Shah AK, Granger DN. Oxygen radicals: Effect on intestinal vascular permeability. *Am J Physiol* 1984; **247**: G167-70.
- 21 Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am J Physiol* 1986; **251**: G567-74.
- 22 Granger DN, Zimmerman BJ, Kvietys PR, Grisham MB. Role of granulocytes in gastrointestinal ischemia. *FASEB J* 1988; **2**: A1268.
- 23 Hernandez LA, Grisham MB, Twohig B, Arfors KE, Harlan JM, Granger DN. Role of neutrophils in ischemia-reperfusion induced microvascular injury. *Am J Physiol* 1987; **253**: H699-703.
- 24 Perry MA, Wadhwa S, Parks DA, Pickard W, Granger DN. Role of oxygen radicals in ischemia-induced lesions in the cat stomach. *Gastroenterology* 1986; **90**: 362-7.
- 25 Smith SM, Grisham MB, Mancini EA, et al. Gastric mucosal injury in the rat: role of iron and xanthine oxidase. *Gastroenterology* 1987; **92**: 950-6.
- 26 Itoh M, Guth PH. Role of oxygen derived free radicals in hemorrhagic shock induced gastric lesions in the rat. *Gastroenterology* 1985; **88**: 1162-7.
- 27 Hiraishi H, Terano A, Ota S, Ivey KJ, Sugimoto T. Oxygen metabolite-induced cytotoxicity to cultured rat gastric mucosal cells. *Am J Physiol* 1987; **253**: G44-8.
- 28 Cross CE, Halliwell B, Allen A. Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. *Lancet* 1984; **i**: 1328-30.
- 29 Grisham MB, Von Ritter C, Smith BF, La Mont JT, Granger DN. Interaction between oxygen radicals and gastric mucin. *Am J Physiol* 1987; **253**: G93-6.
- 30 Adkison D, Hollwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN. Role of free radicals in ischemia-reperfusion injury to the liver. *Acta Physiol Scand* 1986; **126**: 101-8.
- 31 Atalla SL, Toledo-Pereyra LH, Mackenzie GH, Cederna JP. Influence of oxygen-derived free radical scavengers on ischemic livers. *Transplantation* 1985; **40**: 584-90.
- 32 Marubayashi S, Dohi K, Ochi K, Kawasaki T. Role of free radicals in ischemic rat liver cell injury: prevention of damage by α -tocopherol administration. *Surgery* 1986; **99**: 184-91.
- 33 Marubayashi S, Dohi K, Yamada K, Kawasaki T. Changes in the levels of endogenous coenzyme Q homologs, α -tocopherol, and glutathione in rat liver after hepatic ischemia and reperfusion, and the effect of pretreatment with coenzyme Q₁₀. *Biochem Biophys Acta* 1984; **797**: 1-9.
- 34 Siems W, Mielke B, Muller M, Heumann C, Rader L, Gerber G. Status of glutathione in the rat liver. Enhanced formation of oxygen radicals at low oxygen tension. *Biomed Biochim Acta* 1983; **42**: 1079-89.
- 35 Jennische E. Possible influence of glutathione on postischemic liver injury. *Acta Pathol Microbiol Scand* 1984; **92**: 55-64.