

# Antioxidant properties of 5-ASA: Potential mechanism for its anti-inflammatory activity

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**ABSTRACT:** There is a growing body of experimental data to suggest that the inflamed intestine and/or colon may be subjected to considerable oxidative stress. The most probable source of these oxidants are the phagocytic leukocytes, since these cells are present in large numbers in the inflamed mucosa and are known to produce significant amounts of potentially injurious reactive oxygen species in response to inflammatory stimuli. The authors' laboratory and others have demonstrated that 5-aminosalicylic acid (5-ASA) possesses potent antioxidant activity, including free radical scavenging properties and the ability to decompose neutrophilic oxidants (eg, hypochlorous acid) and detoxify hemoprotein-associated oxidizing agents. 5-ASA has the additional property of being able to chelate iron and render it poorly redox active. Therefore, it is proposed that much of the anti-inflammatory activity of 5-ASA may be due to its numerous antioxidant properties. *Can J Gastroenterol* 1990;4(7):295-302

**Key Words:** *Etiology, Intestinal injury, Neutrophils, Oxidants*

## Les propriétés antioxydantes de l'acide 5-aminosalicylique: Mécanisme possible de son activité anti-inflammatoire

**RESUME:** Des données expérimentales de plus en plus nombreuses suggèrent que l'intestin ou le côlon irrité est peut-être assujéti à un stress oxydatif considérable. Il est probable que les oxydants responsables proviennent des leucocytes phagocytaires qui abondent dans la muqueuse enflammée: on sait en effet qu'ils produisent, en réaction aux stimuli inflammatoires, des quantités considérables d'espèces oxygénées réactives potentiellement nuisibles. Les études effectuées en laboratoire, par les auteurs et d'autres chercheurs, démontrent que l'acide 5-aminosalicylique (5-ASA) est un antioxydant puissant; il a la capacité d'éliminer les radicaux libres, de décomposer les oxydants neutrophiles (acide hypochloreux) et de détoxifier l'hémoprotéine associée aux agents oxydants. Le 5-ASA a de plus la propriété de chelater le fer et d'en affaiblir l'action oxydoréductrice. C'est pourquoi il est suggéré que l'action anti-inflammatoire du 5-ASA serait due en grande partie à ses nombreuses propriétés antioxydantes.

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**U**LKERATIVE COLITIS IS A RECURRENT inflammation of the colon and rectum characterized by rectal bleeding, diarrhea, fever, pain, anorexia and weight loss. Active episodes of this disease are characterized by the extravasation and infiltration of large numbers of inflammatory leukocytes (neutrophils, eosinophils, monocytes and macrophages) into the colonic mucosa (1). This enhanced inflammatory infiltrate is accompanied by extensive mucosal injury including edema, crypt abscesses, loss of goblet cells, decreased mucus production, erosions and mucosal ulcerations (1). Oral administration of sulphasalazine has been shown to be very effective in attenuating the mucosal inflammation and injury associated with this disease. Pharmacokinetic studies have demonstrated that sulphasalazine passes through the upper gastrointestinal tract unmodified until it reaches the colon, where it is metabolized by endogenous bacteria to yield 5-aminosalicylic acid (5-ASA) and sulfapyridine. Several clinical studies have demonstrated that 5-ASA is the pharmacologically active moiety of sulphasalazine (2-4).

## PROPOSED MECHANISMS OF ACTION OF 5-ASA

Although sulphasalazine has been used clinically for more than 40 years, the mechanism(s) by which 5-ASA



**TABLE 1**  
**Proposed mechanisms of action of 5-aminosalicylic acid (5-ASA)**

	IC <sub>50</sub> (μM)	Reference
Cyclo-oxygenase inhibitor	10,000	6
Lipoxygenase inhibitor	6000	8
Inhibitor of neutrophil function		
Phagocytosis	NE	9
Chemotaxis	NE	9
Degranulation	NE	9
FMLP binding	>5000	13
Inhibitor of antibody secretion	1050-1350	14
Antioxidant		
Superoxide radical scavenger	10-20	18
Hydroxyl radical scavenger	400-1000	17,37
Carbon-, peroxy- or nitrogen-centred free radical scavenger	5-10	19,PS
Hypochlorous acid scavenger	25-400	17,38
Iron chelator	300	37
Scavenger of hemoprotein-associated oxidants	20-50	38,PS

*In some studies 5-ASA was found to have no effect (NE). The IC<sub>50</sub> of 5-ASA for scavenging peroxy radicals or the hemoglobin-associated free radical was presented in the present study (PS). FMLP n-formyl-methionyl-leucyl-phenylalanine*

protects the mucosa during active episodes of ulcerative colitis remains only speculative. It has been suggested that 5-ASA may exert its protective effect by inhibiting the enzyme cyclo-oxygenase, thus attenuating the formation of potentially pro-inflammatory prostaglandins (5,6). The concentration required to inhibit this enzyme by 50% (IC<sub>50</sub>) was found to be approximately 10 mM (6) (Table 1).

The initial enthusiasm for this mechanism has diminished over the past few years after it was discovered that more potent and specific inhibitors of cyclo-oxygenase may actually exacerbate inflammatory tissue injury. Work by Stenson and colleagues (7,8) has demonstrated that sulphasalazine and to a lesser extent 5-ASA are capable of inhibiting lipoxygenase activity from activated neutrophils. Using leukotriene B<sub>4</sub> synthesis as a measure of lipoxygenase activity, they found that the IC<sub>50</sub>s for sulphasalazine and 5-ASA were approximately 0.8 and 6.0 mM, respectively, when exogenous arachidonic acid was included as the substrate. However, the IC<sub>50</sub>s increased to 2.8 and 6.4 mM, respectively, when endogenous arachidonic acid was used as the substrate (8). The physiological significance of inhibition by the parent drug (sulphasalazine) is presently un-

clear. In addition to inhibition of arachidonate metabolism, high concentrations of sulphasalazine and 5-ASA have been shown to inhibit certain functions of human neutrophils such as migration, degranulation, phagocytosis and superoxide formation (9-12). It has also been determined that high concentrations of sulphasalazine and 5-ASA inhibit the binding of the bacterial peptide, n-formyl-methionyl-leucyl-phenylalanine (FMLP) to human neutrophils, thereby inhibiting FMLP-induced arthritis in rabbits and FMLP-induced oxygen radical formation in vitro (13). A recent report by MacDermott *et al* (14) shows that 5-ASA is effective at inhibiting mitogen-stimulated secretion of immunoglobulins synthesized by peripheral blood and intestinal mononuclear leukocytes. The IC<sub>50</sub> for 5-ASA in this particular study was approximately 1.0 to 1.35 mM (14). Again, as with the studies on neutrophil function, the physiological significance of inhibition of leukocyte metabolism and function using high concentrations of sulphasalazine or 5-ASA is, at the present time, not apparent. More recent work has demonstrated that 5-ASA has potent antioxidant or free radical scavenging properties in vitro, which has been suggested to account for some of its therapeutic efficacy in vivo (15-

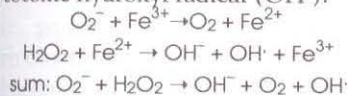
23). A major limitation in defining the protective mechanisms of 5-ASA has been uncertainty regarding the concentration of this anti-inflammatory agent within the colonic mucosal interstitium. The authors recently determined the colonic interstitial concentrations of 5-ASA and its n-acetylated derivative in the healthy feline colon perfused with concentrations of 5-ASA known to result from oral administration of sulphasalazine or from intrarectal administration of 5-ASA preparations. It was found that perfusion of the colonic lumen with 10 mM 5-ASA produced an interstitial concentration of 5-ASA of approximately 100 to 150 μM (24). This represented only 1 to 2% of the total luminal 5-ASA delivered to the mucosa. These studies also revealed that the cat has a diminished capacity to n-acetylate 5-ASA, since only a small percentage (less than 10%) of the drug was detected as n-acetyl-5-ASA in the mucosal interstitium (24). The results of this study appear to have important implications relative to the extrapolation of in vitro experiments regarding proposed mechanisms of action of 5-ASA and their respective IC<sub>50</sub>s (Table 1). For example, studies that implicate inhibition of arachidonate metabolism or leukocyte function require concentrations of 5-ASA greater than 1000 μM – a level far in excess of the mucosal interstitial concentrations determined for the colon. These data appear to minimize the role of 5-ASA as an inhibitor of arachidonate metabolism or leukocyte function. However, it should be noted that the inflamed mucosa may be more permeable to 5-ASA than healthy colon. Interestingly, the IC<sub>50</sub>s of 5-ASA for a variety of antioxidant mechanisms fall within the experimentally determined interstitial concentrations, suggesting that some of the antioxidant properties of 5-ASA may be operative in vivo. If one of the major mechanisms of action of 5-ASA is as an antioxidant or free radical scavenger, then one would predict that the intestinal mucosa may be subjected to significant oxidative stress during active episodes of inflammation. The following section discusses



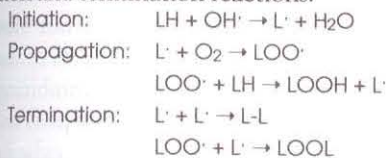
evidence which supports the idea that intestinal inflammation enhances reactive oxygen metabolism.

### REACTIVE OXYGEN METABOLISM DURING INTESTINAL INFLAMMATION

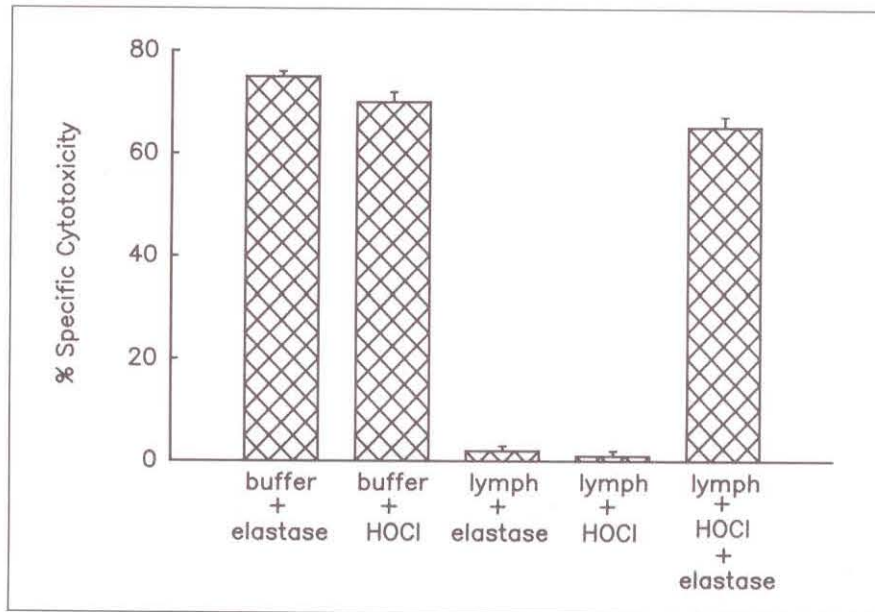
One of the hallmarks of active episodes of colitis is the infiltration of large numbers of phagocytic leukocytes into the mucosal interstitium. Recent evidence suggests that the infiltration of these inflammatory cells may be mediated by the potent pro-inflammatory agents leukotriene B<sub>4</sub> and/or platelet activating factor, since both of these mediators have been shown to be elevated in inflamed mucosa (25,26). Concurrent with this enhanced inflammatory infiltrate is extensive mucosal injury, suggesting that the phagocytes play an active role in mediating some of the mucosal damage associated with this disease. It is known that the interaction of certain pro-inflammatory stimuli (eg, leukotriene B<sub>4</sub>, platelet activating factor) with specific receptors on the neutrophilic plasma membrane results in the production and release of large quantities of reactive oxygen metabolites such as superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Neither superoxide nor hydrogen peroxide are particularly reactive; however, they will interact in the presence of certain transition metals such as iron (Fe) to generate the highly reactive and cytotoxic hydroxyl radical (OH·):



The hydroxyl radical is one of the most potent oxidants produced in biological systems. It is capable of oxidizing and peroxidizing a wide variety of biomolecules such as protein, carbohydrate and lipid (27). Lipid peroxidation is a classical free radical reaction involving initiation, propagation and termination reactions:

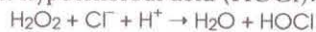


where LH, L·, LOO· and LOOH represent polyunsaturated lipid, lipid alkyl



**Figure 1** Antioxidant and antiprotease activity of intestinal lymph. Pig pancreatic elastase (25 µg/mL) and hypochlorous acid (HOCl) (0.2 mM) were incubated with intestinal epithelial cells (IEC-18;  $2 \times 10^5$  cells per well) in the absence or presence of rat intestinal lymph (50%) for 4 h at 37°C. For some experiments lymph was first exposed to hypochlorous acid (0.2 mM) for 10 mins at 37°C in the absence of intestinal epithelial cells. The solutions containing oxidized lymph and elastase were then added to the cells and incubated for an additional 4 h at 37°C

radical, lipid hydroperoxyl radical and lipid hydroperoxide, respectively. Peroxidation of membrane lipids alters membrane fluidity and may dramatically affect lipid-protein interactions and enzymatic function (27). In addition to the hydroxyl radical, it is known that hydrogen peroxide will interact with hemoglobin to generate a hemo-protein-associated oxidant capable of initiating lipid peroxidation (28). These types of reactions may be important during active episodes of ulcerative colitis because bleeding into the mucosal interstitium will release hemoglobin in proximity to phagocyte-generated hydrogen peroxide (23). In addition to classic reactive oxygen metabolites, activated neutrophils and monocytes also secrete the hemo-protein myeloperoxidase into the extracellular medium where it catalyzes the oxidation of chloride ions by hydrogen peroxide to yield the highly reactive oxidizing and chlorinating agent hypochlorous acid (HOCl).



Hypochlorous acid has been shown to degrade gastrointestinal mucin, enhance mucosal permeability and injure intestinal epithelial cells (29,30). The

authors have recently demonstrated that hypochlorous acid may mediate intestinal epithelial cell injury indirectly by inactivating certain protease inhibitors (alpha-1-protease inhibitor, alpha-2-macroglobulin) found in intestinal interstitial fluid (lymph). Figure 1 shows that intestinal lymph is extremely effective at inhibiting elastase- or hypochlorous acid-mediated cytotoxicity to intestinal epithelial cells in vitro. If, however, lymph is allowed to first interact with physiological levels of hypochlorous acid for 10 mins at 37°C, it loses its ability to protect epithelial cells from a subsequent challenge of elastase. The net result of these reactions in vivo would be uncontrolled, hypochlorous acid-dependent proteolysis.

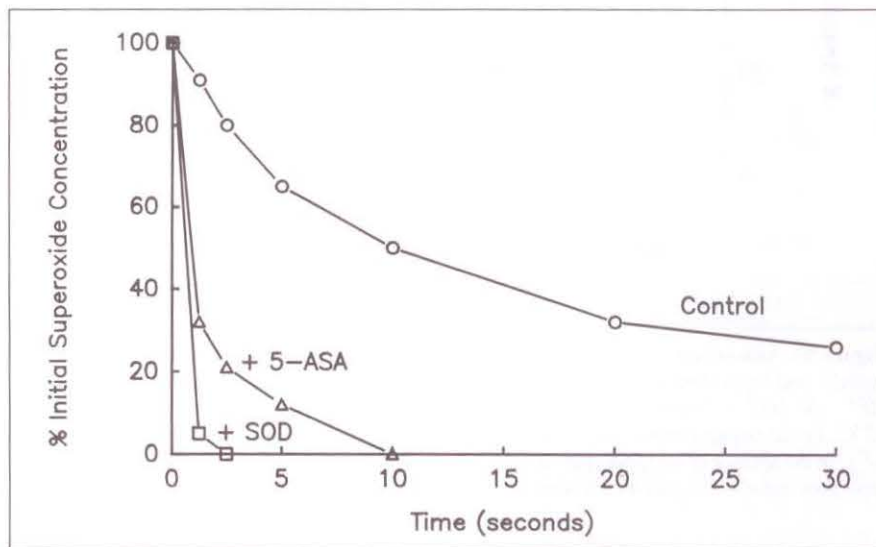
Normally most cells and tissues are protected from the injurious effects of reactive oxygen metabolites by the action of certain antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. However, the present authors have shown that the colonic mucosa contains relatively small levels of superoxide dismutase and catalase compared to other tissues such as the liver (31). Furthermore,



**TABLE 2**  
**Antioxidant enzymes associated with intestinal lymph and epithelial cells**

	SOD (units/mg)	Catalase (units/mg)	GSH (mU/mg)	Protein SH (nmol/mL)
Lymph (feline)	1.0	0.46	6.8	100
IEC-18	26	12	6.1	-

Data represent the means from duplicate determinations and are expressed per mg of protein. IEC-18 Rat intestinal epithelial cells; SOD Superoxide dismutase; GSH Glutathione; SH Sulfhydryl



**Figure 2** Decomposition of the superoxide anion radical mediated by 5-aminosalicylic acid (5-ASA) and superoxide dismutase (SOD). Decomposition of superoxide (potassium superoxide) was assessed by measuring the decrease in absorbance at 250 nm ( $\lambda_{max}$  for superoxide) at pH 9.0 in the absence or presence of superoxide dismutase (20  $\mu$ g/mL) or 5-ASA (0.25 mM)

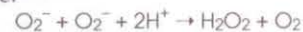
they have found that intestinal interstitial fluid (lymph) is deficient in these enzymes compared to enterocytes (Table 2). The potential for oxidant-induced mucosal injury may be further increased by the enhanced reactive oxygen metabolism determined for granulocytes obtained from patients with active inflammatory bowel disease (32-34). Using chemiluminescence as an indirect measure of active oxygen metabolism, Keshavarzian *et al* (35) have preliminary data to suggest that the inflamed colon produces significantly more active oxygen than does the healthy colon. Taken together, these data suggest that the inflamed colon may be subjected to considerable oxidative stress which would overwhelm the normally low levels of protective enzymes thus leading to mucosal injury. If this hypothesis is correct, then enhancing endogenous antioxidant levels may prove useful in protecting the mucosa during times of

active inflammation. One drug that has potent antioxidant activity is 5-ASA.

#### ANTIOXIDANT PROPERTIES OF 5-ASA

It has been estimated that  $4 \times 10^6$  human neutrophils will consume approximately 20  $\mu$ M oxygen/min when fully activated, and virtually all of this oxygen is converted to superoxide (36). Although superoxide *per se* is not very reactive or cytotoxic it will interact with iron to generate the toxic hydroxyl radical (OH $\cdot$ ). Thus, any compound that decomposes superoxide would attenuate superoxide-dependent formation of hydroxyl radicals. A recent report by Craven *et al* (18) demonstrated that 5-ASA has potent superoxide dismutase-like activity as measured by its ability to inhibit the reduction of cytochrome c by xanthine oxidase-generated superoxide. Using a more direct assay for the decomposition of superoxide - *ie*, the direct

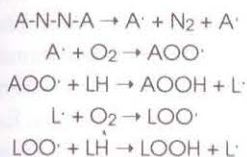
spectrophotometric determination of the superoxide anion radical at 250 nm - it was found that 5-ASA interacts directly with superoxide, causing the rapid decomposition of this radical (Figure 2). The disappearance of superoxide may occur by two possible pathways. One pathway would require the one electron reduction of superoxide by 5-ASA in the presence of protons to yield hydrogen peroxide. The other way in which 5-ASA may decompose superoxide is by the one electron reduction of 5-ASA by superoxide to yield oxygen. Because 5-ASA would acquire an odd electron by either pathway it would become, by definition, a free radical itself. The significance of these observations is currently under investigation in the authors' laboratory. Phagocytic leukocytes also produce large amounts of hydrogen peroxide by the spontaneous or enzymatic (superoxide dismutase) dismutation of superoxide:



The authors have found that 5-ASA reacts very sluggishly with hydrogen peroxide and thus is unlikely to participate in the decomposition of this oxidant *in vivo*. As already mentioned, superoxide and hydrogen peroxide will interact in the presence of iron to yield the highly reactive oxidant hydroxyl radical. This oxidant is capable of mediating the oxidative degradation of a variety of biomolecules, including lipid and carbohydrates. Work from several laboratories including the present authors' has demonstrated that 5-ASA is very effective in scavenging the hydroxyl radical; however, the parent compound sulphasalazine and the metabolically inactive metabolites *n*-acetyl-5-ASA and sulfapyridine are also equally effective (17,37). These data suggest that hydroxyl radical scavenging does not account for the therapeutic action of 5-ASA. In contrast with these studies, the authors have found that 5-ASA - but not sulphasalazine, *n*-acetyl-5-ASA or sulfapyridine - is effective in inhibiting the lipid peroxidation (IC<sub>50</sub> 8  $\mu$ M) initiated by organic peroxy radicals generated from the thermal decomposition of 2,2'-azobis (amidinopropane)

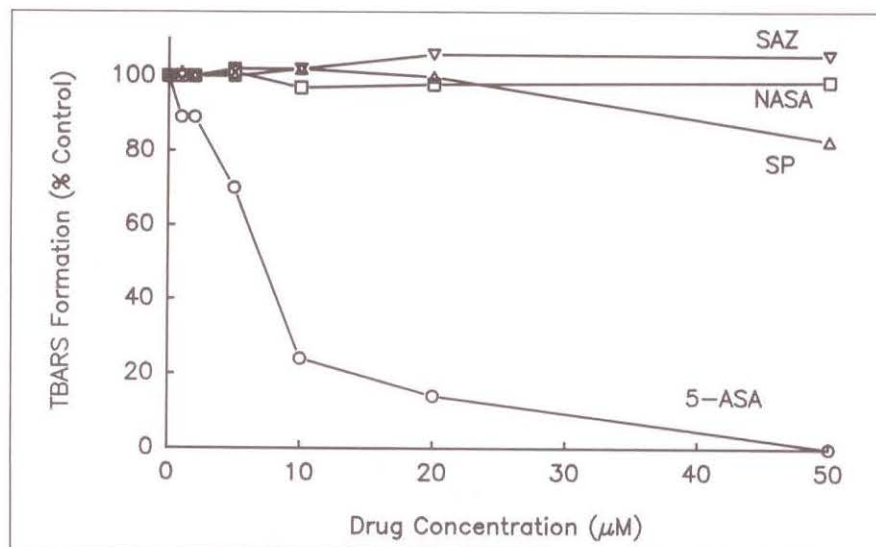


dihydrochloride (A-N-N-A) (Figure 3):

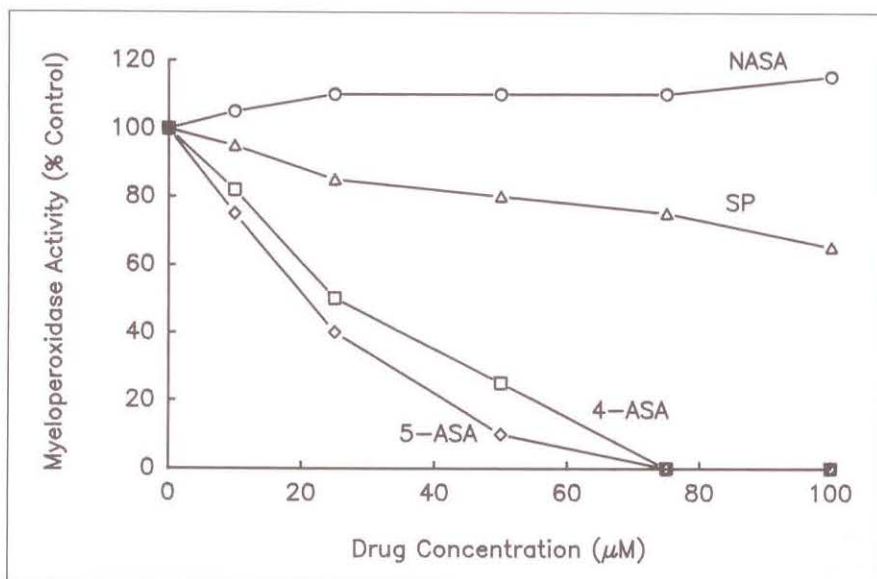


where  $\text{A}^\cdot$ ,  $\text{AOO}^\cdot$  and  $\text{AOOH}$  represent the alkyl radical, peroxy radical and hydroperoxide, respectively. Because lipid peroxidation is not initiated by the superoxide-dependent, iron-catalyzed formation of hydroxyl radical in this system, the inhibitory effect of 5-ASA is due solely to its ability to scavenge peroxy free radicals. These data agree with and extend the findings of Ahnfelt-Ronne and Nielson (19), who demonstrated potent scavenging of a nitrogen-centred free radical by 5-ASA ( $\text{IC}_{50}$  5  $\mu\text{M}$ ) but not by sulphasalazine or sulfapyridine. Another property of 5-ASA that may contribute to its antioxidant activity is its ability to chelate iron. Obviously, any compound capable of binding iron and rendering it poorly redox active would be very effective in inhibiting the formation of hydroxyl radicals. The authors have recently demonstrated that 5-ASA inhibits the iron-catalyzed, hydroxyl radical-mediated degradation of deoxyribose by chelating iron and preventing its interaction with superoxide and hydrogen peroxide ( $\text{IC}_{50}$  300  $\mu\text{M}$ ) (37). *n*-Acetyl-5-ASA and sulphasalazine were only modestly effective, whereas sulfapyridine was inactive, suggesting a relatively selective effect by the therapeutically active metabolite.

Myeloperoxidase-catalyzed oxidation of chloride ions by hydrogen peroxide to yield hypochlorous acid represents another significant pathway of oxidant production in inflamed tissue. Recent work by von Ritter et al (38) demonstrated that 5-ASA, sulfapyridine, *n*-acetyl-5-ASA and sulphasalazine are all very effective at scavenging hypochlorous acid in vitro ( $\text{IC}_{50}$  25 to 30  $\mu\text{M}$ ). However, Aruoma and co-workers (17) have shown that 5-ASA was selective in its ability to protect alpha-1-protease inhibitor against inactivation by hypochlorous acid ( $\text{IC}_{50}$  400  $\mu\text{M}$ ), suggesting that



**Figure 3** Effects of 5-aminosalicylic acid (5-ASA), sulphasalazine (SAZ), *n*-acetyl-5-ASA (NASA), and sulfapyridine (SP) on peroxy radical-mediated lipid peroxidation. Lipid (80% phosphatidyl choline) was incubated with the thermolabile free radical initiator 2,2'-azobis (amidinopropane) dihydrochloride for 30 mins at 37°C in the absence or presence of varying concentrations of sulphasalazine or its metabolites. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid-reactive substances (TBARS)



**Figure 4** Effects of 5-aminosalicylic acid (5-ASA), 4-aminosalicylic acid (4-ASA), *n*-acetyl-5-ASA (NASA) and sulfapyridine (SP) on myeloperoxidase activity. Human myeloperoxidase activity was assessed by measuring the hydrogen peroxide-dependent oxidation of 3,3',5,5'-tetramethylbenzidine in the absence or presence of varying concentrations of sulphasalazine or its metabolites

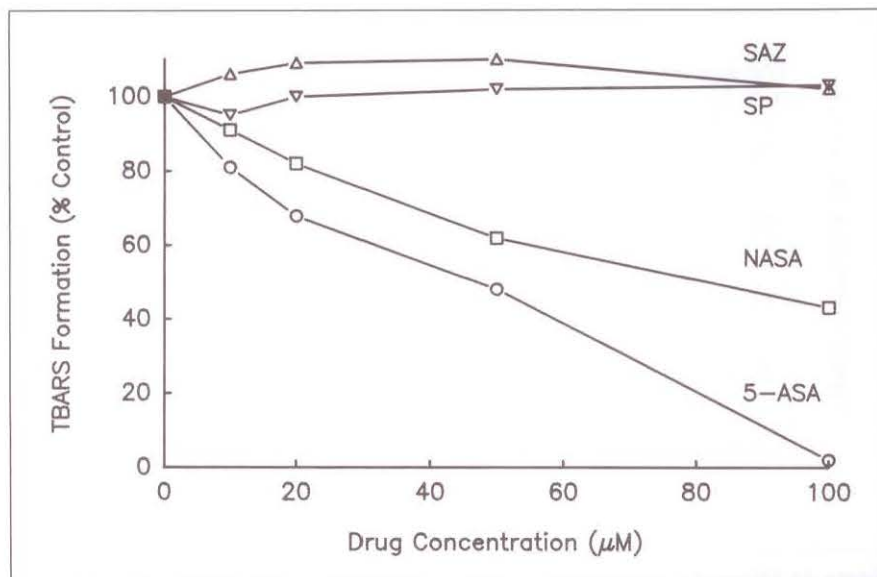
some of the beneficial effects of 5-ASA may be due to its ability to interact selectively with and decompose hypochlorous acid in the presence of other biological compounds. The myeloperoxidase-catalyzed formation of hypochlorous acid requires interaction between hydrogen peroxide and the enzyme to form a potent hemoprotein-

associated oxidant termed 'compound I'. This porphyrin cation radical is a potent oxidizing agent capable of oxidizing a wide variety of biological compounds in addition to chloride ions:

$$\begin{aligned} \text{P-Fe}^{3+} + \text{H}_2\text{O}_2 &\rightarrow \text{P}^\cdot\text{-Fe}^{4+}=\text{O} + \text{OH}^- \\ \text{P}^\cdot\text{-Fe}^{4+}=\text{O} + \text{AH} &\rightarrow \text{P-Fe}^{3+} + \text{A}^\cdot + \text{OH}^- \end{aligned}$$

Where  $\text{P-Fe}^{3+}$ ,  $\text{P}^\cdot\text{-Fe}^{4+}=\text{O}$ ,  $\text{AH}$  and  $\text{A}^\cdot$  represent the hemoprotein, por-





**Figure 5** Effects of 5-aminosalicylic acid (5-ASA), *n*-acetyl-5-ASA (NASA), sulfapyridine (SP), and sulphasalazine (SAZ) on hemoglobin-catalyzed lipid peroxidation. Lipid was incubated in the absence or presence of varying amounts of sulphasalazine or its metabolites with hemoglobin (0.02 mM) and hydrogen peroxide (0.1 mM) for 20 mins at 37°C. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid-reactive substances (TBARS)

pyrin cation radical, substrate and oxidized substrate, respectively.

The present authors have found that 5-ASA and 4-aminosalicylic acid (4-ASA) were much more effective in decomposing compound I of myeloperoxidase – and thus much more potent at inhibiting the activity of myeloperoxidase – than sulfapyridine or *n*-acetyl-5-ASA. IC<sub>50</sub>s of 20 and 25 µM were determined for 5-ASA and 4-ASA, respectively (Figure 4). Apparently 5-ASA and 4-ASA act as alternative substrates for compound I, preferentially becoming oxidized instead of the substrate. It is quite possible that this is the reason why Ahnfelt-Ronne *et al* (21) detected significant levels of oxidation products of 5-ASA in sulphasalazine-treated patients with active inflammatory bowel disease. It has also been suggested that the interstitial hemoglobin released during intestinal bleeding may mediate some of the mucosal injury by interacting with phagocyte-derived hydrogen peroxide to generate ferryl (Fe<sup>4+</sup>) hemoglobin, a hemoprotein-associated free radical similar to myeloperoxidase compound I. Ferryl hemoglobin is a potent oxidant capable of initiating lipid peroxidation (28). The authors have found that 5-ASA – and to a lesser extent sul-

phasalazine or sulfapyridine – selectively inhibit hemoglobin-catalyzed lipid peroxidation by acting as an alternative substrate for ferryl hemoglobin (IC<sub>50</sub> 50 µM) (Figure 5). These types of reactions may help explain observations made by Houlst and Page (39), who demonstrated enhanced production of certain prostaglandins by colonic mucosa in the presence of relatively small concentrations of 5-ASA (0.5 mM). It is known that prostaglandin synthetase contains two enzymatic activities, including cyclo-oxygenase and hemoprotein hydroperoxide peroxidase activities. During the enzymatic reaction there is progressive inhibition of the enzyme due to oxidative inactivation of the hemoprotein peroxidase. It is well known that certain antioxidants (eg, phenolic compounds) inhibit this inactivation process and prolong prostaglandin production. Apparently the lipid hydroperoxide generated by cyclo-oxygenase (prostaglandin G<sub>2</sub>) combines with the hemoprotein peroxidase to generate a compound I-like oxidant. In the absence of an exogenous electron-donating substrate (antioxidant) the hemoprotein-localized free radical oxidizes certain amino acid residues proximal to the active site, which ultimately results in inac-

tivation of the enzyme. It is intriguing to speculate that 5-ASA, by virtue of its antioxidant activity, may protect the mucosa by enhancing the formation of protective prostaglandins such as prostacyclin and/or prostaglandin E derivatives. Indeed recent reports suggest that certain prostaglandin E analogues are potent antiulcer compounds for the colon (40).

## SUMMARY AND CONCLUSIONS

There is a growing body of experimental data to suggest that the chronically inflamed intestine and/or colon may be subject to considerable oxidative stress. The most probable source of these oxidants is the phagocytic leukocytes since these cells are present in large numbers in the inflamed mucosa and are known to produce significant amounts of reactive oxygen species in response to certain inflammatory stimuli. Furthermore, the colonic mucosa contains relatively small amounts of the antioxidant enzymes superoxide dismutase and catalase. If reactive oxygen species play an important role in mediating mucosal injury in inflammatory bowel disease, then it should be possible to attenuate this injury by the use of antioxidants. Indeed, Emerit *et al* (41) have recently demonstrated, in a limited clinical study, that intramuscular administration of superoxide dismutase protected the intestine from inflammatory tissue injury in patients with refractory Crohn's disease. In addition, a variety of antioxidant enzymes (superoxide dismutase, catalase) and scavengers have proven useful in attenuating the microvascular and mucosal injury associated with the acute intestinal inflammation induced by ischemia (42). It may not be a coincidence that the anti-inflammatory metabolite 5-ASA is a potent antioxidant that possesses multiple mechanisms of action including nitrogen-, carbon- and oxygen-centred free radical scavenging properties, and the ability to decompose hypochlorous acid and scavenge hemoprotein-associated oxidants. 5-ASA has the additional property of being able to chelate iron. The reason



that 5-ASA is so effective in vivo may be due to this multitude of antioxidant properties. This would also suggest that other, more potent antioxidants may prove beneficial in the treatment of inflammatory bowel disease.

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