Effect of Luminal Exposure of Oxidants on Intestinal Mucosal Lipid Peroxidation and Absorptive Function

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The lumen of the gastrointestinal tract is likely to have free iron and prooxidants that might generate free radicals, resulting in structural and functional damage to the absorptive cells. In the present study the effect of luminal exposure of different oxidants on intestinal mucosal lipid peroxidation and absorptive function in anaesthetized animals has been studied. Oxidants that require iron for free radical generation did not have any effect on the mucosa, whereas free radicals generated by using 2,2'-azobis (2-amino propane) dihydrochloride or menadione, which do not require iron, resulted in lipid peroxidation as assessed by malondialdehyde and conjugated diene formation and depletion of the antioxidant alpha-tocopherol. These oxidants also induced water and electrolyte secretion as studied by luminal perfusion. This observation is physiologically significant since the presence of prooxidants along with iron in the lumen may not have a damaging effect on the mucosa.

Key words: Intestinal absorption; lipid peroxidation; prooxidants

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The most important function of the gastrointestinal tract is the digestion and absorption of dietary materials. The mucosal permeability of the gastrointestinal tract is of major importance in maintaining the electrolyte balance and host defence against microorganisms and toxic substances. Several pathophysiological conditions have been shown to be accompanied by increases in intestinal mucosal permeability (1-3). Even though the exact cause of this increase in permeability is not known, studies point to oxygen-derived free radicals as one of the possible causes. It has been shown that chloramine, hydrogen peroxide, and other oxidants could damage the mucosa and increase permeability of the intestine (4-6). Oxygen free radicals can react and damage cellular proteins, carbohydrates, nucleotides, and lipids (?). The most important of these is the lipid peroxidation, wherein oxygen-derived free radicals attack the lipids in the membrane and cause peroxidation, resulting in complete destruction of the membrane. Our earlier in vitro studies have shown that the intestinal mucosal cell membranes are resistant to lipid peroxidation (8, 9). Further studies indicated that mucosal cell membranes are resistant to iron-dependent lipid peroxidation induced by H2O2, ascorbate-Fe3++, nico-tinamide adenine dinucleotide phosphate, reduced form (NADPH)-adenosine diphosphate (ADP)-Fe3++, xanthine-xanthine oxidase, whereas they are susceptible to an iron-independent free radical generating system using 2,2'-azobis(2-amino propane) dihydrochloride (ABAP) (10). The intestinal mucosa is constantly in contact with the luminal contents, which include dietary materials, such as transition metals, ascorbic acid, and rancid fat, along with bacterial metabolites, and some of these are prospective prooxidants. Even though damage to the intestinal epithelial cells due to free radical generation has been shown under schaemic and other stress conditions, very little work has been done on the luminal exposure of the intestine to various oxidants. In the present study the intestinal lumen was exposed to various free radical-generating systems in situ, and the damage was assessed by measuring various peroxidation variables and functional alterations.

MATERIALS AND METHODS

Hepes, thiobarbituric acid, Tris, bovine serum albumin, alpha-tocopherol, 1,1,2,3-tetrame thoxypropane, xanthine oxidase, menadione, and PEG 4000 were obtained from Sigma Chemical Co., USA. ABAP was obtained from Poly Sciences Inc., USA. All solvents were distilled before use, and for high-performance liquid chromatography (HPLC) special-grade solvents were used. All other chemicals used were of analytical grade.

Steady-state perfusion of rat intestine in vivo

Wistar rats starved overnight and weighing 180-200 g were anaesthetized with pentobarbitone (50 mg/kg). The abdomen was opened with a midline incision, and a 30- to 40-cm segment of the small intestine was isolated and gently flushed with normal saline. This was cannulated proximally and distally, and the segment returned to the abdomen, and the
incision closed. An isoosmotic solution containing various oxidants in 0.9% NaCl was instilled into the intestinal segment, which was then clamped on both ends. After 30 min of incubation the solution was flushed out, and the intestine was either excised immediately for lipid peroxidation measurements or perfused with 0.9% NaCl containing 2.5 g/l PEG 4000. Perfusion solution at 37°C was infused continuously at a rate of 0.5 ml/min, using a Vickers Medical Tronic IP4 syringe pump. Body temperature was maintained by means of an overhead lamp. After an initial equilibration period of 1 h, three consecutive 10-ml collections of the effluent from the distal cannula were made into glass test tubes. Serial measurement of PEG concentration showed the intestinal segment to be in a steady state during the experimental period. Each segment was perfused with one solution only. PEG recovery during the collection period was 98%. At the end of the experiment the perfused segment was excised immediately, firmly blotted, and weighted. For water and electrolyte absorption experiments, the wet and dry weight of the segments were noted. All solutions instilled in the intestinal lumen were isoosmotic to rat plasma (305±5 mosmol/kg) and pH 7.0. The various oxidants used were all final concentrations, in 0.9% NaCl, pH 7.0, 1 mM H2O2; 2 SAU menadione; 3) 50 mM 2,2′-azois (2-aminopropanol) dihydrochloride; and 4) 1 mM xanthine + 10 μM xanthine oxidase.

Assay methods

Assays for lipid peroxidation were done by measuring the malondialdehyde (MDA), conjugated diene, and alpha-tocopherol levels of the mucosa and were carried out on 10% homogenate of the mucosa in 0.9% NaCl, as described earlier (10). Protein was measured using bovine serum albumin as standard (11). PEG was measured turbidometrically (12) and determined by flame photometry. Calculation of net water and electrolyte movements was done in accordance with a standard formula (13).

RESULTS

Lipid peroxidation variables were assessed in the intestinal mucosal cells after exposure of the luminal side of the intestine to different oxidants and are shown in Fig. 1. Normally, the presence of transition metals such as Fe2+ is essential for generation of oxygen free radicals by certain oxidants, but our earlier in vitro studies have suggested that intestinal mucosa is resistant to iron-dependent generation of free radicals. Hence in the present in vivo study oxidants used include both iron-dependent systems such as oxidine-xanthine oxidase and H2O2 and iron-independent systems such as ABAP and menadione. Among the oxidants used, ABAP and menadione showed generation of MDA, whereas other oxidants did not show any significant change compared with control. Conjugated diene is yet another product of lipid peroxidation, and quantitation of this in the mucosa after exposure to oxidants showed that the presence of ABAP, menadione, or xanthine-xanthine oxidase in the lumen resulted in the formation of conjugated diene. Alpha-tocopherol is a lipid-soluble antioxidant that protects the cell membranes from lipid peroxidation. Free radicals oxidize tocopherol to quinone, and disappearance of alpha-tocopherol is an indication of the extent of lipid peroxidation. A decrease of nearly 75% of the alpha-tocopherol was observed when the mucosa was exposed to ABAP, and a 28% decrease was observed in the presence of H2O2. Other oxidants did not have any significant effect.

Lipid peroxidation might lead to functional alterations in the cell. The absorptive capacity of the mucosa was assessed after exposure to various oxidants. As can be seen in Fig. 2, there was an increased passage of water and electrolytes into the lumen (secretion) on exposure to ABAP and menadione. The net water secretion was 31 ± 7 and 16 ± 3 μl/min/g wet weight on exposure to ABAP and menadione, respectively. The sodium secretion was 5.16 ± 0.97 and 3.16 ± 0.48 μM/min/g wet weight with ABAP and menadione exposure, respectively. This suggested an alteration in the permeability properties of the intestinal mucosa.
properties of the mucosal membrane on exposure to oxidants. Other oxidants did not alter secretory or absorptive properties of the mucosal cells.

DISCUSSION

Oxygen-derived free radicals have been suggested to play an important role in the pathologic conditions associated with ischaemia/reperfusion injury (14-17) and inflammation (18, 19) of the gastrointestinal tract. The intestinal mucosa is unique in that free radicals could be formed both in the tissue and in the lumen. Although various pathologic conditions associated with free radicals in the mucosa are due to their formation in the tissue, very little is known about the mucosal damage on exposure of the lumen to oxidants. This is important since the presence of prooxidants derived from diet, drugs, and bacterial metabolites might generate free radicals in the lumen. One of the mechanisms by which free radicals damage the cells is through lipid peroxidation, and during this process tissue antioxidants, such as alpha-tocopherol, are depleted. A measure of the lipid peroxidation products such as malonaldehyde and conjugated diene and antioxidant tocopherol level is an indication of the extent of peroxidation. In the present report, the luminal side of the intestinal mucosa was exposed to various oxidants in a live animal, and its effects on mucosal lipid peroxidation and absorptive function were studied.

The luminal presence of oxidants such as ABAP or menadione indicated the occurrence of lipid peroxidation in the mucosa, whereas other free radical-generating systems such as H₂O₂ or xanthine-xanthine oxidase did not generate any lipid peroxidation products. Most of the free radical-generating systems require the presence of free iron, but ABAP could generate free radicals by thermal decomposition and does not require iron for this process. The present results indicate that free radicals generated independent of iron might lead to peroxidation of the mucosa, and this in vivo observation is similar to our earlier in vitro observation using mucosal membranes (8-10).

The present study also looked at the effect of oxidant exposure on intestinal absorptive/secretory function. Similar to lipid peroxidation, intraluminal exposure with ABAP or menadione produced a significant increase in the secretion of water and electrolytes into the lumen, and other oxidants studied did not have any effect. These results suggest the following: 1) lipid peroxidation of the mucosa is associated with functional alteration, leading to luminal secretion of water and electrolytes, and 2) free radical generation and lipid peroxidation can occur only with oxidants that do not require the presence of free iron. Several intestinal pathophysiologic conditions are associated with an increase in mucosal permeability and electrolyte secretion. In ischaemia/reperfusion injury, in which a role for free radicals has been suggested, an increase in vascular permeability has been shown (20, 21). Studies have shown that hypoxanthine, a substrate for xanthine oxidase, accumulates in ischaemic tissues as a result of adenosine triphosphate catabolism. The reducible substrate, molecular oxygen, is provided with reperfusion. The oxygen radicals produced by this enzyme-substrate system play a major role in the damage to intestinal
tissue and endothelial cells under these conditions. It was also shown that superoxide generated by the hypoxanthine-xanthine oxidase system increases mucosal albumin clearance (22). The present study also used the xanthine-xanthine oxidase system but could not demonstrate lipid peroxidation or electrolyte secretion. This may be because less xanthine oxidase was used in the present study for the generation of free radicals. This observation suggests that xanthine oxidase, when present in the gut lumen, may not be harmful to the tissue, but when it is present in the circulation, such as in ischemia/reperfusion, it induces free radical damage. Tissue generation of free radicals in inflammation leads to formation of chloramines, and this has been shown to evoke electrolyte and water secretion in the intestine (23, 24).

Presence of free iron facilitates the formation of active oxygen free radicals. In normal physiologic systems iron is always associated with proteins such as ferritin or transferrin, and iron in the free form is not present. Whereas in certain pathologic conditions the availability of free iron makes the tissue undergo lipid peroxidation damage. An exception to this is the lumen of the intestine, where dietary iron is present in free form and is available for free radical generation. It has been suggested that free fatty acids associated with the mucosal microvilli may form a complex with iron, and this complex is incapable of inducing peroxidation (25). The present in vivo experiments confirm our earlier in vitro observation that iron-induced lipid peroxidation may not occur in the mucosa. This observation is significant, since the simultaneous presence of free iron and prooxidants in the lumen may not be harmful to the mucosa.

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