LUMINAL EXPOSURE OF OXIDANTS ALTER COLONIC ABSORPTIVE FUNCTION

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Summary

The colonic lumen is likely to contain oxidants derived from unabsorbed dietary materials including transition metals, rancid fat, drugs and bacterial metabolites. The present study looks at the effect of luminal exposure of different oxidants on colonic mucosal lipid peroxidation and absorptive function. All the oxidants tested induced fluid and electrolyte secretion and indomethacin, a prostaglandin synthesis inhibitor reversed this effect. Oxidants did not induce mucosal lipid peroxidation. This study suggests that oxidants induce functional alterations in colon possibly through stimulation of prostaglandin generation without influencing mucosal lipid peroxidation.

Key Words: oxidants, colonic secretion, prostaglandin, lipid peroxidation

The colonic mucosa plays an important role in maintaining proper fluid and solute exchange as well as providing a barrier to translocation of potentially harmful bacteria and bacterial metabolites. The enhanced mucosal permeability that results from injury to epithelium affects electrolyte balance and causes other functional alteration (1). Colonic epithelium is known to be damaged in a variety of pathophysiological conditions such as ulcerative colitis and ischemic colitis (2). Reactive oxygen metabolites are some of the important effector molecules in acute inflammation. It is known that nucleic acids, proteins and membrane lipids are the targets of these oxyradicals (3). It has been suggested that oxidants may contribute to the diarrhea associated with inflammatory conditions (4). Colonic lumen may contain unabsorbed dietary materials including possible prooxidants such as transition metals, drugs and rancid fat which

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might contribute to the generation of free radicals. In addition to this, colonic lumen has large number of resident bacteria and these bacterial metabolites which include $\text{H}_2\text{O}_2$ produced by catalase negative bacteria might form an important source of free radicals. Hence the colonic mucosa is likely to be exposed to oxidants present in the lumen. The present study looks at the possible damaging effect of luminal oxidants on colonic cells by instillation of different oxidants into the lumen of anaesthetised rat.

**Materials and Methods**

Menadione, cumene hydroperoxide (CUOOH), tertiary butyl hydroperoxide (t-BUOOH), xanthine oxidase (XO), xanthine (X), indomethacin and bovine serum albumin (BSA), various lipid standards and alpha tocopherol were all obtained from Sigma Chemical Co. All other chemicals used were of analytical grade.

**Perfusion of rat colon in vivo**

Overnight fasted rats weighing 150-200 g were anaesthetised with pentobarbitone (50 mg/kg body weight). The abdomen was opened with a midline incision and the large intestine was isolated. A cannula was inserted in the colon 2cm from the caecum and another one was placed 3cm proximal to the anal verge. The colonic contents were removed by gently flushing with Krebs Hansleit (KH) buffer. The segment was returned to the colon and incision closed. An iso-osmotic solution containing various oxidants in KH buffer was instilled separately in to the colonic segment which was clamped at both ends. After 30 min incubation, the solution was flushed out and the colon was either excised immediately for biochemical estimation or perfused with buffer containing 2.5 gm/l polyethylene glycol (PEG) 4000. Perfusion was performed at a flow rate of 0.5 ml/min using a Vickers Medical Treonic IP4 syringe pump. Body temperature was maintained by means of overhead lamp. After an equilibration period of 1 hr, three consecutive 10 min fractions of the effluent were collected from the distal cannula. (The total duration of PEG 4000 infusion was 90 min). PEG recovery during the collection period was 98%. At the end of the experiment, the perfused segment was excised, firmly blotted and weighed. For water and electrolyte absorption experiments, wet and dry weight of the colon was noted. In all the experiments control rats were incubated for 30 min with buffer alone and for absorption studies control rats were perfused with PEG 4000 after the incubation as in the treated rats. The various oxidants used were 100 μM menadione, 1mM hydrogen peroxide (H$_2$O$_2$), 100 μM tertiary butyl hydroperoxide (t-BUOOH), 5 mM ascorbic acid+5μM FeSO$_4$ (Ascorbate-Fe"$^+$") or 1mM xanthine+10munits xanthine oxidase (X-XO) (all final concentrations). PEG content of the perfusate was measured turbidimetrically (5). Sodium was measured using flame photometer and chloride was measured using chloride titrator. Net water and electrolyte absorption was calculated using standard formula (6). Net absorption was expressed as positive values and net secretion as negative values. One group of animals received indomethacin, a prostaglandin synthesis inhibitor (10mg/kg bodyweight) IP, 30 min prior to incubation with various oxidants.
Biochemical estimations
Assays for lipid peroxide, conjugated diene and alpha tocopherol
were carried out using 10% homogenate of the mucosa in KH buffer.
Lipid peroxide was estimated by thiobarbituric acid reaction (7).
Total lipid was extracted from the mucosal homogenate by Bligh
and Dyer’s method (8) which was dried using nitrogen and
dissolved in 1 ml of heptane. Conjugated diene was estimated at
233 nm and calculated using molar extinction coefficient of 2.52
x 10^4. Alpha tocopherol was estimated by HPLC (9) after
extraction as described for liver microsomes (10). Protein
content was measured using BSA as standard (11). Extracted
lipids were used for quantitation of neutral and phospholipids.
Neutral lipids were separated by TLC using silica gel G plates
with solvent system hexane:diethyl ether:acetic acid (80:20:1).
Separated lipids were visualized by exposure to iodine and
individual spots were scraped, eluted with chloroform:methanol
(2:1 v/v) and dried under nitrogen. Cholesterol was estimated as
described (12). Di and tri glycerides were quantitated
colorimetrically (13). Nonesterified fatty acids were separated
and quantitated after methylation using gas chromatography.
Fatty acids were separated on a 5% EGGS-X column and
heptadecanoic acid was used as internal standard. Total
phospholipids were estimated (14) after acid digestion.

Statistics
The data were analysed for the presence of significant
differences among the experimental and control groups by
student’s t test.

Results
Fig. 1 shows the lipid peroxidation parameters in colonic
mucosa after oxidant exposure. No significant change in the
level of malonaldehyde or conjugated diene was seen in
experimental groups as compared to the control. Alpha
tocopherol, an antioxidant which protects cells from oxidant
injury was decreased significantly after exposure to menadione
(p<0.001) and H_2O_2 (p<0.05) whereas other oxidants did not change
its level in the mucosa. Fig. 2, 3 and 4 shows the secretory
properties of the colonic mucosa after luminal exposure to
oxidants. All the oxidants studied induced fluid and sodium
secretion in to the lumen (p<0.01 for all the oxidants used when
compared with the control in Fig. 2A and p<0.001 for all oxidants
when sodium absorption was compared with the control in Fig. 3A).
Except X-XO and ascorbate-iron, all other oxidants induced
chloride secretion and with these two oxidants chloride
absorption was reduced considerably as compared to control (Fig.
4A, P<0.05 for X-XO and ascorbate-iron and p<0.001 for all the
other oxidants). Prior administration of indomethacin reversed
the net water secretion induced by luminal exposure of oxidants
(Fig. 2B). A similar effect was seen in sodium and chloride
secretion in indomethacin treated rats after exposure to oxidants
as shown in Fig. 3B and 4B. Colonic mucosal lipid analysis
showed no alteration in the content and composition after
exposure to oxidants (Table 1).
Colonic mucosal level of lipid peroxide, conjugated diene and alpha tocopherol after luminal exposure to oxidants. Level of alpha tocopherol was decreased significantly when exposed to menadione (p<0.001) and H₂O₂ (p<0.05).

A. Water absorption by the colon after luminal exposure to various oxidants. All oxidants used induced water secretion when compared with the control (p<0.01). Positive values represent net water absorption and negative values represent net water secretion. B. Influence of indomethacin on net water absorption after exposure to various oxidants.
A. Sodium absorption by the colon after exposure to various oxidants. All the oxidants used induced sodium secretion when compared to control (P<0.001). Positive values represent net sodium absorption and negative values represent net sodium secretion. B. Effect of indomethacin on net sodium absorption after exposure to various oxidants.

A. Chloride absorption by the colon after luminal exposure to various oxidants. Chloride absorption was reduced by oxidants when compared to control. p <0.05 for X-XO and ascorbate-iron. For all the other oxidants used p <0.001. Positive values represent net chloride absorption and negative values represent net chloride secretion.

B. Effect of indomethacin on chloride absorption after exposure to various oxidants.
<table>
<thead>
<tr>
<th></th>
<th>CHOLESTEROL</th>
<th>PHOSPHOLIPID</th>
<th>CHOLESTEROL ESTER</th>
<th>TRIGLYCERIDE</th>
<th>DIGLYCERIDE</th>
<th>FREE FATTY ACID</th>
</tr>
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<tbody>
<tr>
<td>CONTROL</td>
<td>41.6 ± 0.86</td>
<td>288 ± 8.10</td>
<td>2.3 ± 0.40</td>
<td>29.1 ± 4.30</td>
<td>9.5 ± 0.75</td>
<td>34.1 ± 9.81</td>
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<tr>
<td>MENADIONE</td>
<td>44.5 ± 5.12</td>
<td>279 ± 6.30</td>
<td>1.7 ± 0.01</td>
<td>19.9 ± 7.14</td>
<td>9.9 ± 0.34</td>
<td>30.3 ± 3.20</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>57.5 ± 5.12</td>
<td>260 ± 16.40</td>
<td>2.7 ± 0.09</td>
<td>32.6 ± 0.86</td>
<td>7.7 ± 0.17</td>
<td>39.1 ± 14.60</td>
</tr>
<tr>
<td>CuO₂H</td>
<td>46.0 ± 0.03</td>
<td>254 ± 7.40</td>
<td>2.0 ± 0.01</td>
<td>21.4 ± 1.16</td>
<td>7.1 ± 1.21</td>
<td>25.7 ± 0.74</td>
</tr>
<tr>
<td>t. BUOOH</td>
<td>46.2 ± 5.40</td>
<td>247 ± 14.14</td>
<td>1.6 ± 0.19</td>
<td>26.2 ± 4.14</td>
<td>6.5 ± 0.39</td>
<td>33.1 ± 3.64</td>
</tr>
<tr>
<td>Ascorbate-Fe²⁺</td>
<td>39.2 ± 0.42</td>
<td>278 ± 3.00</td>
<td>2.5 ± 0.05</td>
<td>27.1 ± 0.98</td>
<td>6.9 ± 0.18</td>
<td>37.9 ± 13.50</td>
</tr>
<tr>
<td>X - XO</td>
<td>37.1 ± 5.69</td>
<td>245 ± 25.00</td>
<td>1.7 ± 0.38</td>
<td>20.01 ± 0.53</td>
<td>8.3 ± 0.35</td>
<td>33.1 ± 3.60</td>
</tr>
</tbody>
</table>
Discussion

Oxygen free radicals have been implicated in many inflammatory disorders including those of the gastrointestinal tract (15,16). Involvement of reactive oxygen species in the pathophysiology of various forms of enteritis and gastric ulceration has been suggested (17). Experimental evidences indicate a role for oxygen free radicals in the damage during ischemia/reperfusion injury to the intestine including the alteration in mucosal permeability and one of the sources for these active species is the infiltrated phagocytes (18, 19). Free radicals damage cells through lipid peroxidation and a measure of peroxidation products such as malonaldehyde and conjugated diene indicates the extent of peroxidation. Extensive lipid peroxidation might lead to membrane disorganisation and reduction in membrane fluidity (20). Modification of lipid composition and or lipid fluidity has been shown to affect the activity of numerous membrane bound proteins including transport proteins (21). Our study has shown that luminal exposure to oxidants did not induce mucosal lipid peroxidation and the lipid composition of the colonic mucosa was not altered. Our earlier in vitro study with isolated colonic cell membranes has shown that these membranes are resistant to lipid peroxidation as assessed by various parameters (22). Although menadione and H$_2$O$_2$ exposure did not induce lipid peroxidation, these oxidants did reduce the level of tocopherol in the mucosa which may be due to the direct oxidation by these oxidants.

Colonic epithelium plays an important role in fluid and electrolyte absorption. It also contributes to the fluid loss in diarrheal condition by its secretory mechanism. In ulcerative colitis, mucosal inflammation is associated with marked impairment of colonic sodium and water absorption (23). Fluid secretion has been shown in ulcerative colitis and in colon exposed to dihydroxy bile acids and fatty acids (24). It is likely that in inflammatory conditions, free radicals generated by the infiltrated phagocytes may have a role in colonic fluid secretion. In the present study, exposure of colonic lumen to oxidants resulted in fluid secretion and all the oxidants tested showed this effect. Our earlier study has shown that in the case of small intestine, menadione exposure leads to fluid and electrolyte secretion whereas other oxidants had no significant effect (25). This suggests that colonic and small intestinal epithelium shows functional differences in response to oxidants present in the lumen. Actual mechanism by which oxidants stimulate water and electrolyte secretion is not known. Sodium is actively transported from the lumen which provides osmotic force for the movement of water. Na$^+$K$^+$ ATPase is the biochemical equivalent of the epithelial sodium pump. Inhibition of this enzyme may be a possible mechanism in the pathogenesis of sodium and water malabsorption. In an earlier study, H$_2$O$_2$ was shown to inhibit Na$^+$K$^+$ATPase activity and this effect was prevented by d-alpha tocopherol (26). Another possible mechanism by which free radicals interfere with sodium transport is by oxidising sulfhydryl groups in sodium channel proteins (27). The main group of agents that alter intestinal electrolyte transport include neurotransmitters, hormones, bacterial enterotoxins and immune system. Cyclooxygenase products of arachidonic acid
metabolism have been shown to play a central role in mediating this effect. In vitro studies using Ussing chambers has shown that H₂O₂ can stimulate colonic electrolyte secretion and a role for prostaglandins in this process was suggested (28). Indomethacin, an inhibitor of prostaglandin synthesis has been shown to promote absorption in the small intestine (29, 30). This compound also suppressed prostaglandin mediated colonic secretion induced by interleukin 1 in rats (31). This study has shown that prior administration of indomethacin reverses the oxidant-induced water and electrolyte secretion effect which suggests a role for prostaglandins in oxidant-induced fluid and electrolyte secretion in the colon. Recent in vitro work in colon suggests that both prostaglandins and the enteric nervous system may play an important role in oxidant mediated chloride secretion (32). Raised concentrations of prostaglandins were found in inflamed colonic mucosa, serum, urine and stools of patients with active ulcerative colitis (33). The mechanism of action of prostaglandins remains to be determined. This study suggests that fluid and electrolyte secretion in colon during oxidant exposure may not be mediated by lipid peroxidation.

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References