Surgical Stress Induces Phospholipid Degradation in the Intestinal Brush Border Membrane

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Submitted for publication June 12, 2000
Published online October 30, 2000

Background. Surgical stress can lead to translocation of bacteria from the intestine into the systemic circulation. The intestinal brush border membrane (BBM) plays an important role in defense against such invasion by luminal bacteria and endotoxin. Our earlier work has shown the development of oxidative stress in the intestine after surgical stress and since the BBM is sensitive to free radical attack, this study examined the effect of surgical stress on the structure and function of intestinal BBM.

Methods. Intestinal BBM were isolated from control and after surgical stress and compared for structural and functional alterations. Surgical stress was also carried out following pretreatment with the xanthine oxidase inhibitor allopurinol or the nitric oxide donor L-arginine, to study the protection offered by these compounds.

Results. Surgical stress affected intestinal BBM structure as well as function. A decrease in alkaline phosphatase activity and a-tocopherol content, accompanied by an increase in lipid peroxidation, was seen. Surgical stress induced phospholipid degradation with generation of arachidonic acid. Functional impairment with a decrease in glucose transport ability was also seen. These changes are prevented by inhibition of xanthine oxidase by allopurinol pretreatment but not by NO.

Conclusion. Surgical stress in the small intestine causes structural and functional alterations in the BBM through oxidative stress. This damage could affect gut barrier integrity and generation of arachidonic acid might mediate distal organ dysfunction.

Key Words: surgical stress; BBMV; phospholipase.

INTRODUCTION

Enterocytes are highly polarized cells consisting of a well-defined brush border membrane (BBM) and a basolateral membrane. The brush border is the site of final digestion of food materials as well as absorption of nutrients [1]. It also acts as a barrier that excludes luminal bacteria from the internal environment. Under certain pathological conditions, this barrier is adversely affected due to permeability changes in the intestine that can be brought about by a variety of conditions, including burn trauma and surgical stress [2]. Our earlier work has shown that oxidative stress plays a role in surgical stress-induced damage to the intestine [3]. These include widening of intercellular spaces in the intestinal mucosa and permeability alterations, as well as altered mitochondrial function. This damage was maximum 60 min after stress and returned to control by 24 h. Inhibition of the superoxide-generating enzyme, xanthine oxidase (XO), protects against this damage, indicating the importance of oxygen free radicals in this process [4].

It has been shown that transport of glucose, amino acids, and inorganic phosphate across the intestinal brush border membrane is by a secondary active process [5] and alteration in the lipid composition and physical state of the membrane lipids can alter membrane-bound enzyme activities and transport systems [6]. Membrane lipids and proteins are the targets of free radicals and an important mechanism of cell damage by these active species is lipid peroxidation. Biological membranes are readily susceptible to peroxidative damage and it has been shown that lipid...
peroxidation alters the fluidity of rat liver plasma membrane and certain tumor cell membranes [7] and also decreases glucose transport [8]. Infection by Salmonella typhimurium is accompanied by peroxidative damage of BBM and a decrease in the level of vitamin E and protein thiol [9]. Nitric oxide (NO) is an important molecule whose biological effects are under intense investigation. NO modulates G.I. function and plays a role in repair of mucosal injury and regulation of intestinal mucosal barrier function [10].

The present study looks at the effect of surgical stress on structural and functional alterations in the intestinal brush border membrane and the protection offered by inhibition of the superoxide-generating enzyme, XO, by allopurinol or the nitric oxide donor, L-arginine.

Materials and Methods

Tris(hydroxymethyl)aminomethane (Tris), N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic acid] (Hepes), bovine serum albumin (BSA), p-nitrophenyl phosphate, peroxidase, O-dianisidine, glucose oxidase, γ-glutamyl-p-nitroanilide, glycylglycine, thiobarbituric acid (TBA), L-leucine p-nitroanilide, allopurinol, L-arginine, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), and lipid standards were obtained from Sigma Chemical Co. (St. Louis, MO). Polyethylene glycol (PEG) 4000 was obtained from Fluka AG (Switzerland). 3H-labeled glucose was obtained from Bhabha Atomic Research Center (Bombay, India). Millipore membranes (pore size 0.45 μm) were obtained from Millipore (India). All other chemicals used were of analytical grade.

Allopurinol and L-arginine pretreatment. For inhibition of XO activity, rats were given intraperitoneal injection of allopurinol (100 mg/kg body weight), 1 h before induction of surgical stress. For L-arginine pretreatment, animals were injected with L-arginine (300 mg/kg body weight) intraperitoneally, 30 min before surgery.

Induction of surgical stress. Surgical stress was induced as described [3]. Briefly overnight fasted rats were anesthetized by ketamine injection (50 mg/kg body weight, ip). The abdominal wall was opened by a vertical incision of approximately 4 cm. The intestine was gently moved and the ileocecal junction identified. The intestine was then gently handled along its entire length from the ileocecal junction proximally, simulating the “inspection” that occurs in a clinical setting. The intestine was then replaced back in the abdominal cavity and the whole process was completed within 1 to 2 min. Following this, the abdominal wall was sutured and the animals were killed by decapitation, 60 min after the surgical procedure. Our earlier study has shown that maximum damage to the intestine due to oxidative stress occurs 60 min following surgical stress [3]. Allopurinol- or L-arginine-pre-treated rats were also subjected to surgical stress and their intestines used for further studies. For control, rats were killed by decapitation without opening the abdominal wall after injecting ketamine or L-arginine or allopurinol. This study was cleared by the Institutional Animal Experimentation Ethics Committee.

Isolation of brush border membranes. BBM were isolated from small intestine of control and surgically stressed rats by the PEG precipitation method. Briefly, the luminal contents were washed thoroughly with ice-cold saline and the mucosa was scraped using a glass slide. Approximately 3% homogenates of mucosa were prepared in 2 mM Tris-HCl containing 50 mM mannitol, pH 7.1, using a Potter-Elvehjem homogenizer for 2–3 min at full speed. This was allowed to remain at 4°C for 15 min and filtered using nylon cloth. To this, a 50% PEG solution was added to make a final concentration of 10% PEG, stirred for 15 min, and centrifuged at 7500g for 15 min. The pellet was discarded and the supernatant was spun at 27,000g for 40 min. To the pellet, 30 ml suspension buffer (10 mM Tris-HCl and 300 mM mannitol, pH 7.1) was added and centrifuged at 27,000g for 40 min. The pellet was washed twice with the same suspension buffer and finally suspended in 1 ml of the same buffer using a syringe fitted with a 26-gauge needle. Purity of the isolated BBM was checked by enrichment of the marker enzyme alkaline phosphatase (ALP), sucrase, and maltase. Protein was estimated using bovine serum albumin as standard [11].

Enzyme assays. Activities of ALP [12], sucrase [13], maltase [13], γ-glutamyl transferase [14], leucine aminopeptidase [15], total ATPase [16], and Mg2+ ATPase [16] were assayed as described. The enzyme activity is expressed as units per milligram of protein (units are expressed as mmol/min/mg protein for ALP and μmol/min/mg for maltase, sucrase, total ATPase, and Mg2+ATPase).

Peroxidation parameters. Malonaldehyde (MDA) was measured using the TBA method [17]. The amount of MDA formed was calculated from the standard curve prepared using 1,1,3,3-tetramethoxypropane and values were expressed as nanomoles per milligram of protein. For conjugated diene measurement, total lipids from BBM were extracted as described [18], dissolved in 1 ml heptane and the peroxidation products produced were measured using the TBA method [17]. The peroxidation activity of BBM in control and after surgical stress was calculated from the standard curve prepared using 1,1,3,3-tetramethoxypropane and values were expressed as nanomoles per milligram of protein (units are expressed as mmol/min/mg protein for ALP and μmol/min/mg for maltase, sucrase, total ATPase, and Mg2+ATPase).

![Graph showing enzyme activity](https://via.placeholder.com/150)

**FIG. 1.** Alkaline phosphatase activity of BBM in control and after surgical stress with and without allopurinol or L-arginine pretreatment. (Each value represents mean ± SD of three separate estimations. *P < 0.05 when compared to control. #P < 0.05 when compared to surgical stress.)
Diacylglycerol 65.41
Cholesterol ester 5.07
Total thiol (nmol/mg protein) 27.25

described [26]. Briefly 50 for their ability to transport glucose by uptake measurements car-
filter was counted using a LKB Rack-Beta scintillation counter.

and transferred to counting vials. The radioactivity retained in the
10 mM Hepes, pH 7.5) and immediately filtered under constant
mixture was diluted with 2 ml of ice-cold stop buffer (150 mM NaCl,
(mg protein) 0.02 0.01# 0.37 ± 0.15#

mM NaSCN, 50
malandehyde (nmol/mg protein) 1.26 0.3 0.46 ± 0.3* 0.3 ± 0.01#

Statistical analysis. Data are expressed as means ± SD. Statisti-
cal analysis was performed with Student’s t test and the Bonferroni
correction for multiple t test was applied where necessary.

RESULTS

The brush border membrane plays a primary role in
digestion and absorption. To assess surgical stress-
induced damage, the activities of membrane-
associated enzymes were measured in BBM isolated
from control and surgically stressed rat small intest-
tine. The specific activity of sucrase, maltase, 
- glutamyl transpeptidase, and leucine aminopepti-
dase remained unaltered after surgical stress (Table 1)
whereas ATPase activity showed a significant de-
crease. A decrease in alkaline phosphatase activity was
also seen after surgical stress (Fig. 1). Our earlier work
has shown that surgical stress results in activation of enterocyte XO leading to oxidative stress and it is
known that nitric oxide can offer protection to the
intestine against damage. Hence surgical stress was
carried out on rats pretreated with and without allo-
purin or L-arginine and changes in BBM structure
and function were compared with control. As seen in
Fig. 1, pretreatment with allopurinol and L-arginine
and function were compared with control. As seen in
Fig. 1, pretreatment with allopurinol and L-arginine

| TABLE 2 |
| BBM Oxidative Stress Parameters in Control and after Surgical Stress with and without Allopurinol or L-Arginine |
| Control | Surgical stress | Allopurinol + surgical stress | L-Arginine + surgical stress |
| Malanaldehyde (nmol/mg protein) | 1.26 ± 0.07 | 2.85 ± 0.09* | 1.3 ± 0.13# |
| Conjugated diene (µmol/mg protein) | 0.3 ± 0.02 | 0.46 ± 0.3* | 0.3 ± 0.01# |
| Total thiol (nmol/mg protein) | 27.25 ± 1.01 | 27.26 ± 1.24 | 28.64 ± 1.59 |
| α-Tocopherol (ng/mg protein) | 169 ± 10.6 | 84.2 ± 7.22* | 194.2 ± 9.08# |

Note. Each value represents mean ± SD of three separate estimations.
P < 0.05 when compared to control.
P < 0.05 when compared to surgical stress.

TABLE 3

Intestinal BBM Neutral Lipid Composition in Control and after Surgical Stress with and without Allopurinol or L-Arginine Pretreatment

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Control</th>
<th>Surgical stress</th>
<th>Allopurinol + surgical stress</th>
<th>L-Arginine + surgical stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>64.40 ± 4.81</td>
<td>60.28 ± 1.59</td>
<td>70.54 ± 4.99</td>
<td>70.26 ± 3.74</td>
</tr>
<tr>
<td>Cholesterol ester</td>
<td>5.07 ± 0.45</td>
<td>4.75 ± 0.28</td>
<td>6.11 ± 0.38</td>
<td>5.55 ± 0.37</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>113.95 ± 10.28</td>
<td>111.15 ± 4.69</td>
<td>103.3 ± 5.19</td>
<td>100.51 ± 5.1</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>65.41 ± 4.72</td>
<td>67.72 ± 5.53</td>
<td>62 ± 3.02</td>
<td>58.6 ± 5.16</td>
</tr>
</tbody>
</table>

Note. Each value represents mean ± SD of three separate estimations.
of XO inhibition and NO donor were assessed. An increase in MDA and conjugated diene and a decrease in \( \alpha \)-tocopherol content were seen in BBM following surgical stress, which were prevented by allopurinol and \( \ell \)-arginine pretreatment. There was no change in protein thiol content of BBM following surgical stress (Table 2).

Lipids form the backbone of membranes, and to assess structural damage, BBM lipids were analyzed after surgical stress in the absence and presence of XO inhibition and \( \ell \)-arginine. As shown in Table 3, there was no alteration in the neutral lipid composition of BBM after surgical stress. However, a significant alteration in certain phospholipids was seen, with a decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and an increase in lysophosphatidylcholine (lysoPC and lysoPE) (Fig. 2). There was no alteration in other phospholipids after surgical stress (data was not shown). These alterations were prevented by pretreatment with allopurinol but \( \ell \)-arginine did not show any significant effect. These changes in phospholipid composition were associated with an increase in free fatty acids. Table 4 shows the total and individual free fatty acids of BBM after surgical stress with and without pretreatment with allopurinol or \( \ell \)-arginine. As can be seen, the total free fatty acid content is increased after surgical stress as compared to control, with significant two- and fourfold increases in levels of polyunsaturated fatty acids such as linoleic acid and arachidonic acid, respectively. This was prevented by allopurinol treatment while \( \ell \)-arginine pretreatment did not have any effect. Functional integrity of BBM was assessed by measuring the glucose transport ability of the membrane after surgical stress with and without pretreatment with allopurinol or \( \ell \)-arginine. Surgical stress results in a decrease in glucose transport ability of the membrane, which was significant when compared to that of control (Fig. 3). This damage was prevented by allopurinol pretreatment while \( \ell \)-arginine had no significant effect.

**Table 4**  
Free Fatty Acid Composition of BBM in Control and after Surgical Stress with and without Allopurinol of \( \ell \)-Arginine Pretreatment

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control (nmol/mg protein)</th>
<th>Surgical stress (nmol/mg protein)</th>
<th>Allopurinol + surgical stress (nmol/mg protein)</th>
<th>Arginine + surgical stress (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (12:0)</td>
<td>2.64 ± 0.22</td>
<td>2.97 ± 0.07</td>
<td>3.39 ± 0.28</td>
<td>3.18 ± 0.3</td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>2.29 ± 0.18</td>
<td>2.58 ± 0.2</td>
<td>2.45 ± 0.22</td>
<td>3.16 ± 0.26</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>35.12 ± 3.43</td>
<td>46.99 ± 2.22*</td>
<td>34.95 ± 1.59#</td>
<td>44.81 ± 3.81</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>36.04 ± 2.31</td>
<td>45.44 ± 3.4#</td>
<td>33.42 ± 1.56#</td>
<td>37.97 ± 2.02#</td>
</tr>
<tr>
<td>Oleic acid (18:1)</td>
<td>5.29 ± 0.67</td>
<td>11.01 ± 1.16*</td>
<td>7.14 ± 0.41#</td>
<td>13.73 ± 1.41</td>
</tr>
<tr>
<td>Linoleic acid (18:2)</td>
<td>3.28 ± 0.22</td>
<td>13.44 ± 0.27*</td>
<td>3.94 ± 0.31#</td>
<td>12.51 ± 0.69</td>
</tr>
<tr>
<td>Total free fatty acids</td>
<td>84.66 ± 7.02</td>
<td>122.43 ± 7.32</td>
<td>85.29 ± 4.37</td>
<td>115.36 ± 8.49</td>
</tr>
</tbody>
</table>

Note. Each value represents mean ± SD of three separate estimations.

* \( P < 0.05 \) when compared to control.

\# \( P < 0.05 \) when compared to surgical stress.
DISCUSSION

Surgery at remote locations affects intestinal mucosal structure and function. Using a simple model of surgical stress, our earlier work has shown that laparotomy followed by mild intestinal handling results in oxidative stress by activation of xanthine oxidase and mitochondrial dysfunction. This effect is seen maximum at 60 min after surgical stress and reversed to normal by 24 h. In this study we have looked at the structural and functional alterations to the BBM following surgical stress. These membranes are mainly involved in the absorptive process and are also in direct contact with luminal contents. Thus, any damage to these membranes might lead to altered absorption of nutrients. Since XO activation has been shown to be an important factor in surgical stress-induced damage to the intestine, we have looked at the changes in BBM after pretreatment with allopurinol, a known inhibitor of XO. NO has been shown to have a beneficial effect on the intestine under damaging conditions. Kubes et al. have shown that NO modulates epithelial permeability in the feline small intestine [27] and release of NO following gut injury could contribute to functional repair of the epithelial barrier [28]. Hence, we have also looked at the effect of l-arginine, the nitric oxide synthase substrate, which can generate NO, on surgical stress-related damage to the intestine. Our results indicate that among the brush border enzymes, ALP activity is decreased following surgical stress, which was prevented by pretreatment with allopurinol and l-arginine. ALP activity is known to be affected by oxygen free radicals [29] and protection by allopurinol suggests that superoxide generated by XO is responsible for decrease in activity. However, since the NO synthase substrate, l-arginine, also shows a protective effect, a role for NO in this process cannot be ruled out. Similar to other membranes, lipids are important constituents of BBM and are susceptible to free radical attack and reactive oxygen species can damage membrane lipids by peroxidation [30]. It was seen that surgical stress increased MDA and conjugated diene content and decreased α-tocopherol content of BBM, which was prevented by allopurinol and l-arginine pre-treatment. On the other hand there was no change in protein thiol content after surgical stress. It is likely that protein thiols are deeply buried inside the membrane and are not easily accessible to free radical attack. Lipid analyses indicated a degradation of PC and PE with a concomitant increase in lysoPC and lysoPE after surgical stress. In association with these changes, there was also an increase in the level of free fatty acids, especially linoleic and arachidonic acids. These alterations in lipid composition were prevented by allopurinol but not l-arginine pretreatment. Lysophospholipids are cytotoxic and also remodeled to yield
platelet-activating factor (PAF) [31], while arachidonic acid is an important precursor for a number of cellular mediators such as eicosanoids. Generation of arachidonic acid and other fatty acids could be due to activation of phospholipase, possibly phospholipase A2, since there was alteration in phospholipids without any change in neutral lipids [32].

Oxygen free radicals are known to activate phospholipases and we have earlier shown activation of phospholipase A2 (PLA2) in liver mitochondria by superoxide, which brings about alteration in the composition of mitochondrial lipids [30]. Increased lysoPC and lysoPE was evident following lipid peroxidation of PLA2-containing liposomes and microsomes [32]. Activation of PLA2 has been suggested as a pivotal step in the pathogenesis of local injury following intestinal PLA2 has been suggested as a pivotal step in the pathogenesis of local injury following intestinal injury and may therefore play a role in posttrauma events downstream to arachidonic acid formation following surgical stress. This results in altered lipid composition through activation of phospholipase A2, generating arachidonic acid. These changes can be prevented by inhibiting XO using allopurinol. Arachidonic acid generated in the intestine is likely to be the precursor of bioactive lipids which can act as mediators of distal organ dysfunction. The molecular mechanism of PLA2 activation and events downstream to arachidonic acid formation following surgical stress are now under investigation.

In conclusion, this study has shown that surgical stress can bring about structural and functional alterations in the intestinal BBM through oxidative stress. This results in altered lipid composition through activation of phospholipase A2, generating arachidonic acid. These changes can be prevented by inhibiting XO using allopurinol. Arachidonic acid generated in the intestine is likely to be the precursor of bioactive lipids which can act as mediators of distal organ dysfunction. The molecular mechanism of PLA2 activation and events downstream to arachidonic acid formation following surgical stress are now under investigation.

REFERENCES
