Effect of ischemia/reperfusion on intestinal brush border membrane lipid composition, fluidity and enzyme activities

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Oxygen-derived free radicals are known to be generated during ischemia/reperfusion injury and biomembranes are the prime target of these active species. In order to study the effect of \textit{in vivo} generated free radicals on intestinal mucosal membrane, brush border membranes (BBM) were isolated from rat small intestine after subjecting to ischemia (I) and ischemia/reperfusion (I/R) injury and their lipid composition and marker enzyme activity were compared with BBM prepared from control animals. No significant alteration in the lipid composition of BBM was observed after I or I/R as compared to control. Membrane fluidity measurements showed that I/R increased the fluidity of BBM. Activity of alkaline phosphatase, one of the marker enzymes for BBM was reduced by I or I/R whereas activity of another BBM enzyme, sucrase was not altered. The decrease in alkaline phosphatase activity was more after reperfusion. \textit{In vitro} fluidization of BBM using benzyl alcohol indicated that the inactivation of alkaline phosphatase was not due to change in fluidity. These results suggest that free radicals generated during I/R inactivate BBM alkaline phosphatase partially without altering the membrane lipid composition.

The small intestine is vulnerable to I/R injury which is characterized by altered vascular and mucosal permeability\textsuperscript{1-2}. The mechanism of mucosal damage is not clearly known. Among the many suggestions put forward to explain mucosal injury, a role for intraluminal pancreatic protease has been indicated\textsuperscript{3}. Recent studies indicate that oxygen-derived free radicals generated by infiltrated phagocytes play an important role in the mucosal damage in I/R injury\textsuperscript{4}. One of the mechanisms by which free radicals damage the membrane is by lipid peroxidation. The results of morphological, functional and biochemical studies indicate that damage to the cell membrane is the early feature of ischemic injury\textsuperscript{5}. It has been shown that the biochemical basis of membrane alteration in liver, kidney and myocardial ischemia may be related to an accelerated degradation of membrane phospholipids\textsuperscript{6-8}.

Intestinal BBM plays an important role in digestion and absorption of nutrients. Membrane lipids are involved in the modulation of some of the membrane associated function and any change in the membrane lipid or its physical state might alter the membrane function. The present study looks at the physical and chemical alterations to the BBM after I/R, with a view to examine whether the altered mucosal permeability observed in the small intestine after I/R injury could be due to alteration in the membrane lipid composition.

\textbf{Materials and Methods}

Standard lipids and fatty acids, $p$-nitrophenyl phosphate, glucose oxidase, 1,6-diphenyl-1,3,5-hexatriene (DPH), pyrene and bovine serum albumin were all obtained from Sigma Chemical Co. All other chemicals used were of analytical grade.

Development of 60 min ischemia followed by 5 min reperfusion on overnight fasted rats were carried out as described\textsuperscript{9}. Control rats underwent the same procedure without occlusion of superior mesenteric artery. At the end of appropriate time periods, the intestine was removed and the mucosa scraped using a glass slide. Brush border membranes (BBM) were prepared by Kessler's method\textsuperscript{10}. The membranes were washed and suspended in 0.9% NaCl and after leaving a portion for enzyme activity measurements, rest of the membranes were subjected to lipid extraction immediately. Purity of the membrane preparation was assessed by the marker enzymes sucrase\textsuperscript{11} and alkaline phosphatase\textsuperscript{12}. Protein was estimated using bovine serum albumin as standard\textsuperscript{13}.

Lipid analysis—Lipid were extracted by the method of Bligh and Dyer\textsuperscript{14}. Neutral lipids were separated on silica gel G plates using the solvent system $n$-hexane:diethylether:acetic acid (80:20:1

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Results

This study compared the lipid composition and fluidity of intestinal BBM isolated from control and after I/R injury. BBM were prepared by Ca²⁺ precipitation method and their marker enzymes, lipid composition and fluidity were compared. A 16-18 fold enrichment of the marker enzymes, alkaline phosphatase and sucrase were observed in the isolated BBM as compared to the homogenate.

The effect of I and I/R on specific activity of BBM marker enzymes is shown in Table 1. Ischemia (60 min) followed by reperfusion (5 min) decreased the specific activity of alkaline phosphatase without altering sucrase activity. Alkaline phosphatase activity was further decreased after reperfusion as compared to control. However the enrichment and the percentage recovery (30-32%) were same suggesting that the BBM isolated from control, I and I/R of rat small intestine remained same. Table 2 shows the lipid composition of BBM isolated from control, I and I/R. There was no change in the major lipid content or composition due to I and I/R. Free fatty acids were the major component of BBM lipids.

Table 1—Comparison of marker enzymes after ischemia/reperfusion (I/R) in rat small intestinal brush border membranes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
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<tbody>
<tr>
<td>Alkaline Phosphatase</td>
<td>Homogenate</td>
<td>0.17±0.03</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td></td>
<td>BBM</td>
<td>3.02±0.03</td>
<td>0.78±0.02</td>
</tr>
<tr>
<td>Sucrease</td>
<td>Homogenate</td>
<td>0.15±0.03</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td></td>
<td>BBM</td>
<td>2.85±0.34</td>
<td>0.77±0.01</td>
</tr>
</tbody>
</table>

*p < 0.05 as compared to control. *p < 0.01 as compared to control.

Table 2 —Lipid composition of rat small intestinal brush border membranes after ischemia/reperfusion (I/R)

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>Ischemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>173±14.9</td>
<td>169±06.4</td>
<td>177±12.0</td>
</tr>
<tr>
<td>Total phospholipids</td>
<td>320±08.9</td>
<td>329±07.1</td>
<td>315±10.0</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>243±05.2</td>
<td>223±11.0</td>
<td>239±06.0</td>
</tr>
<tr>
<td>Dgllycerides</td>
<td>28±04.1</td>
<td>38±06.2</td>
<td>28±04.4</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>52±06.5</td>
<td>53±08.1</td>
<td>48±12.6</td>
</tr>
</tbody>
</table>
Analysis of individual phospholipids revealed no significant alteration in the composition both in control and after I and I/R injury (data not shown). The physical state of membrane lipids were evaluated using the lipid soluble probes, DPH and pyrene. As shown in Table 3 there was an increase in fluidity after I/R as evidenced by decreased fluorescence anisotropy of DPH and increased excimer/monomer ratio of pyrene. The decrease in alkaline phosphatase activity could be due to the alteration in membrane fluidity and this was checked by in vitro fluidizing the isolated BBM by treatment with benzyl alcohol. Table 4 shows the effect of benzyl alcohol on BBM fluidity and alkaline phosphatase activity. Although benzyl alcohol decreased BBM fluidity, it did not alter the activity of alkaline phosphatase.

**Discussion**

Earlier histological study has shown that 60 min ischemia of the small intestine causes decrease in mucosal thickness, shortening of the villi and lifting of the villi from the basement membrane. The damage was severe in the case of ischemia followed by 5 min reperfusion. The molecular mechanism involved in the mucosal damage in I/R injury is not yet established. In the present study, the effects of I/R on BBM marker enzymes and lipid composition have been investigated.

The specific activity of alkaline phosphatase was reduced in mucosal homogenate as well as in the purified BBM from I and the decrease was more in I/R.

**Table 3—Effect of ischemia and reperfusion on DPH anisotropy and excimer/monomer ratio of pyrene in rat small intestinal brush border membranes**

<table>
<thead>
<tr>
<th>Condition</th>
<th>DPH Anisotropy</th>
<th>Pyrene Excimer/ Monomer Ratio</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.235 ± 0.005</td>
<td>0.178 ± 0.017</td>
</tr>
<tr>
<td>Ischemia</td>
<td>0.226 ± 0.011*</td>
<td>0.203 ± 0.030*</td>
</tr>
<tr>
<td>Ischemia/Reperfusion</td>
<td>0.203 ± 0.008*</td>
<td>0.227 ± 0.025*</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01 and ***p < 0.001 as compared to control.

**Table 4—Effect of benzyl alcohol on DPH anisotropy and alkaline phosphatase activity in rat small intestinal brush border membranes**

<table>
<thead>
<tr>
<th>Condition</th>
<th>DPH Anisotropy</th>
<th>Alkaline Phosphatase Activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.241 ± 0.002</td>
<td>2.92 ± 0.14</td>
</tr>
<tr>
<td>+ Benzyl alcohol</td>
<td>0.215 ± 0.006*</td>
<td>3.02 ± 0.25</td>
</tr>
</tbody>
</table>

*p < 0.01 as compared to control.

rats as compared to the control. A similar decrease in the activity of renal BBM alkaline phosphatase after I has been reported. Earlier studies have indicated that oxygen-derived free radicals play an important role in mediating the damage in I/R injury. Infiltrated phagocytes are responsible for generating free radicals and this results in damage to the surrounding mucosa. In vitro studies have shown inactivation of isolated rat intestinal BBM alkaline phosphatase on exposure to oxygen free radicals. The free radical mediated inactivation of alkaline phosphatase appears to be due to oxidative damage to the metal ion binding site. It is likely that during I/R, generation of free radicals may be responsible for partial inactivation of the BBM alkaline phosphatase.

Alteration of membrane lipid composition may be responsible for membrane dysfunction. Earlier studies suggested that phospholipase activation plays an important role in the membrane damage during I/R5,6,8. The degradation of membrane phospholipid is due to the activation of phospholipases by calcium. It is known that I/R results in increased calcium influx due to altered membrane calcium permeability and this activates membrane bound phospholipases resulting in degradation of membrane phospholipids. Free fatty acids and lysophospholipids are all membrane active agents with detergent like properties and can cause membrane lysis. Oiamri et al. found that I/R of small intestine increases the activity of phospholipase and decreases lysophospholipase resulting in the accumulation of lysophospholipids22 which was prevented by phospholipase inhibitors. These studies were carried out using whole scraped mucosa which consists of different types of cells including infiltrated phagocytes. In the present study, isolated BBM were used and analysis of BBM lipids after I/R did not show any significant change as compared to the control. Recently it has been shown that pretreatment with nonspecific phospholipase inhibitors failed to prevent mucosal damage during I/R24. Moreover it is known that intestinal BBM phospholipases are calcium independent.

The physical state of the membrane lipids play an important role in modulating the membrane function. This can be determined by measurement of the fluidity changes in the membrane. In the present study, increase in BBM fluidity was observed after I/R injury as compared to the control. A similar increase in membrane fluidity was observed in the case of renal I/R25. The cause for this alteration may involve changes in the membrane lipids, especially phospholipids and cholesterol. In the present study, I/R did not alter the phospholipid composition of the
membrane suggesting that phospholipases may not be involved in fluidity change. The free radicals generated during I/R may alter membrane fluidity through lipid peroxidation. There are reports of both decreased and increased membrane fluidity of isolated membrane after lipid peroxidation.27-19. Earlier work has shown that intestinal BBM are resistant to in vitro lipid peroxidation due to the presence of considerable amounts of free fatty acids. Moreover it is known that small intestinal I/R failed to induce lipid peroxidation. Free radical mediated alteration of membrane proteins and cytoskeletal proteins might also change membrane fluidity. Benzyl alcohol is known to increase the fluidity of biological membranes and thereby modulate certain membrane protein mediated activities. The inability of benzyl alcohol to influence alkaline phosphatase activity suggests that the partial inactivation of alkaline phosphatase in I/R injury may not be due to fluidity change. The alteration of membrane fluidity in I/R may affect the functional properties of BBM. Reports have shown reduced BBM D-glucose and organic cation transport in the case of renal I/R.

Acknowledgement

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