Histopathologic changes during mesenteric ischaemia and reperfusion

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Summary

The basic electrical rhythm (BER) of the intestine is known to decrease during mesenteric ischaemia. Some studies have reported the relationship between the BER and the pathologic changes that occur in the bowel during vascular injury. However, these changes have not been completely elucidated. This study describes the histopathologic pattern when the rabbit small intestine was subjected to ischaemia of varying time lengths (30 – 150 minutes) and subsequent reperfusion for six hours. Intestinal biopsies were taken at baseline, at the end of ischaemia, and at hourly intervals during reperfusion. Microscopic examination of the biopsies revealed evidence of progressive infarction of the mucosa during ischaemia. There was an acute worsening of the pathology during reperfusion, the severity being greater when reperfusion was preceded by longer periods of ischaemia. These changes were statistically significant. The observed pattern in this study shows clearly that reperfusion injury is reflected in the histopathologic response and that this is worse in severity than the response to ischaemia. Studies of longer duration should further clarify the picture during recovery in ischaemia/reperfusion injuries of the bowel.

Keywords: Mesenteric, Ischaemia, Reperfusion, Pathology.

Résumé

Le rythme électrique normal (Basic Electrical Rhythm, BER) de l'intestin diminue pendant la deficiency de sang. Certaines études ont relevé une relation entre le rythme électriques normal (BER) et les changes pathologiques qui se produisent dans la cavité intestinale lors de l'injury vasculaire. Néanmoins ces changes n'ont pas été complètement éclaircis. Cette étude décrit le type histopathologique quand l'intestin grêle du cobaye était soumis à la deficiency pendant un temps varié (30 – 150mn) et la reperfusion souscrite de six heures.

Les biopsies intestinales étaient portées à la baseline à la baseline à la fin de la deficiency et à intervalles d'une heure durant la reperfusion. L'examen microscopique des biopsies révèle l'évidence d'infarction progressive de mucouse durant la deficiency. Il y avait une déoloration accrue de la pathology durant la reperfusion la severeity était plus grande que quand la reperfusion était précédée par une plus longue période de deficiency. Ces changes étaient significants statistiquement. Le modèle observé dans cette étude montre clairement que l'injury de reperfusion est reflecte dans la réponse histopathologique et que ça s'empire en severeity que dans la réponse à la deficiency. Les études d'une durée plus longue peuvent clarifier l'image durant le rétablissement de l'injury de la deficiency reperfusion de la cavity intestinale.

Introduction

The basic electrical rhythm (BER) is an electrical slow wave that is present all the time in the gastrointestinal tract. Studies have shown that the BER decreases during mesenteric ischaemia. The pathologic changes in the bowel during ischaemia have also been correlated to the change in electrical activity of the intestine. The pathologic changes range from mucosal infarction in early ischaemia to mural necrosis after prolonged ischaemia. It is also known that when the vascular supply is restored after ischaemic injury, return of the electrical activity of bowel function is impaired to varying degrees. However, the accompanying histopathologic changes have not been completely elucidated. Knowledge of the changes that take place will be of value in correlating the histopathology with other parameters that are under investigation in the evaluation and monitoring of bowel viability during mesenteric ischaemia. Electrical activity of the intestine is a sensitive index of health and function of the gut. Biomagnetic fields that are related to the electrical activity of the bowel can also be measured as a means of detecting changes in bowel viability. Electrical and magnetic field measurements of the intestine have been compared and found to have very good correlation. Therefore, full histopathologic description and subsequent correlation with electrical and magnetic field measurements will be of value in investigations aimed at the development of non-invasive means of detecting intestinal ischaemia. Acute mesenteric ischaemia is attended by high morbidity and mortality rates, which are due largely to delay in diagnosis. Therefore, non-invasive methods that can be used for early detection of mesenteric ischaemia will be useful. The purpose of this study was to critically look at and describe the histologic changes that occur during ischaemia of varying lengths of time and during a defined period of subsequent reperfusion.

Materials and methods

Adult male New Zealand rabbits (number = 25, and weighing 3 to 4kg) were divided into five groups, A, B, C, D, and E. Each group consisted of five rabbits. After an overnight fast, the rabbit was anaesthetized. General anesthesia was induced by using acepromazine (0.5mg/kg), xylazine (3mg/kg) and ketamine (40mg/kg), and subsequently maintained with ketamine. Intravenous access was established in an ear vein. This was used for administration of drugs (ketamine, heparin) and for administration of saline given to maintain the hydration status of the rabbit. The rabbit was placed on a heating pad and the trunk was covered with a cellophane blanket to maintain its temperature, which was periodically monitored by using a rectal thermometer. After accessing the abdomen via a mid-line incision, a long segment of ileum was identified and isolated by ligating and transecting it proximally and distally. Its mesentery and segmental vascular supply was kept intact. The transection of the gut segment was done to prevent intramural blood supply from subjacent bowel segments, thus ensuring that the only supply was via the isolated segmental blood vessels. The segmental blood supply was checked at baseline before any further manoeuvres, using a Doppler flow probe (Koven Technology, Inc., model ES-100SFM). Biopsy of the gut segment was done.

*Correspondece

WAND Vol. 22 No. 1, January - March, 2003
before inducing ischaemia. Intravenous heparin (125 u/kg) was administered 15 minutes before induction of ischaemia, and subsequently every four hours, to prevent thrombosis of the segmental blood vessels. A balloon occluder, placed around the segmental blood supply and filled with saline, was used to induce ischaemia. The Doppler flow probe was used to confirm that there was no flow of blood distal to the point of occlusion of the segmental vascular supply. The bowel segment was replaced in the abdomen. The abdomen was then closed and covered with the blanket. Different lengths of ischaemia were maintained for the rabbit groups (groups A = 30; B = 60; group C = 90; D = 120; and E = 150 minutes). Biopsy of the test segment of ileum was taken at the end of ischaemia. The balloon of the occluder was then emptied so that blood supply was re-established to the test segment. Blood flow was again confirmed using the Doppler flow probe. Each group of rabbits was subsequently subjected to six hours of continuous reperfusion. Biopsies of the reperfused ileal segment were taken every hour during the whole period of reperfusion. The abdomen was opened via the mid-line wound and closed each time a biopsy was taken. The rabbit was euthanized at the end of the experiment.

Haematoxylin and eosin sections of the biopsies were examined microscopically by a pathologist who had no knowledge of the specimen identity. Using a modification of the Swerdlov and Antonioli system,11 the biopsies were graded on scale of 0 to 6 (no pathologic change to transmural necrosis. Table 1). The pathology scores were recorded as mean ± SEM and analysed to determine statistical significance. The paired t-test (within groups) and the Kruskal-Wallis test (non-parametric one-way analysis of variance, between groups) were used, with significance defined as p<0.05. The values were plotted against time of ischaemia and period of reperfusion.

Results
The mean histologic grades are shown in Table 2 for each ischaemia group at baseline at the end of ischaemia and at the end of 1-hour and 6-hour reperfusion. In group A (30-minute ischaemia) there was no pathologic change detected at the end of ischaemia (grade 0). In group B and C (60 and 90 minutes ischaemia) there was only focal infarction of the mucosa (grade 1). In group B through to group E (60 to 150 minutes ischaemia) there was a linear progression (R² = 0.89) in the worsening of the pathologic grade from grade 1 to grade 2 during ischaemia. This is illustrated in Figure 1. However, there was no statistical difference in the values.

In Figure 2, the mean histologic grades for all groups are plotted against time of end of ischaemia and all time points during reperfusion. Again, for group A the score was grade 0 at baseline and at the end of ischaemia. For group B there was no significant difference in the histologic picture (variable mucosal infarction) at end of ischaemia and at all time points of reperfusion. However, in groups C, D and E there was acute worsening of the pathologic grade from one hour through six hours of reperfusion. This was particularly noticeable at two hours of reperfusion, and by four hours of reperfusion the

### Table 1: Histopathologic scoring based on a modification of the Swerdlov and Antonioli system

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No pathologic change</td>
</tr>
<tr>
<td>1</td>
<td>Focal loss of surface epithelium</td>
</tr>
<tr>
<td>2</td>
<td>Mucosal infarction</td>
</tr>
<tr>
<td></td>
<td>• extensive loss of surface epithelum</td>
</tr>
<tr>
<td></td>
<td>• loss of variable amounts of lamina propria</td>
</tr>
<tr>
<td></td>
<td>• sparing of basal glands</td>
</tr>
<tr>
<td></td>
<td>• intact muscularis mucosae</td>
</tr>
<tr>
<td>3</td>
<td>Submucosal infarction</td>
</tr>
<tr>
<td></td>
<td>• variable necrosis of submucosa</td>
</tr>
<tr>
<td></td>
<td>• complete mucosal necrosis</td>
</tr>
<tr>
<td></td>
<td>• intact muscularis mucosae</td>
</tr>
<tr>
<td>4</td>
<td>Mural infarction</td>
</tr>
<tr>
<td></td>
<td>• loss of muscularis mucosae</td>
</tr>
<tr>
<td></td>
<td>• complete necrosis of mucoa and submucosa</td>
</tr>
<tr>
<td>5</td>
<td>Mural infarction</td>
</tr>
<tr>
<td></td>
<td>• involvement of inner layer of muscularis propria</td>
</tr>
<tr>
<td></td>
<td>• complete necrosis of mucoa and submucosa</td>
</tr>
<tr>
<td>6</td>
<td>Transmural necrosis of entire bowel wall</td>
</tr>
</tbody>
</table>

Fig. 1: Histologic grades of all experimental group at the end of ischaemia. The groups were subjected to ischaemia for different period of ischaemia: A = 30; B = 60, C = 90, D = 120, E = 150 minutes. There was a linear progression in the pathologic changes (R² = 0.89). The histologic grades for B, C, D and E were not statistically different, min = minutes.

Fig. 2: Mean histologic grades in each group (A,B,C,D, E) plotted against time of ischaemia and reperfusion. There was no observed pathologic change at the end of ischaemia in group A. Following ischaemia of long periods in groups C, D, and E, the acute worsening of pathology during reperfusion (grade 4 from 1 hour through 6 hours of reperfusion) was significant (p<0.05, Kruskal Wallis test). The pathologic changes were marked at 2 hours and 4 hours. The error bars have been omitted from the graph to allow for clarity of the illustration. hr = hour; reperf = reperfusion; min = minutes; isch = ischaemia
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Table 2 Histopathology of the bowel at the end of ischaemia of varying periods and at different time points during perfusion.

<table>
<thead>
<tr>
<th></th>
<th>Mean histologic grades ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td>A</td>
</tr>
<tr>
<td>Length of Ischaemia</td>
<td></td>
</tr>
<tr>
<td>30mins</td>
<td>60</td>
</tr>
<tr>
<td>At Baseline</td>
<td>0</td>
</tr>
<tr>
<td>At end of Ischaemia</td>
<td>0</td>
</tr>
<tr>
<td>After 1 hour of reperfusion</td>
<td>1±0.33</td>
</tr>
<tr>
<td>After 6 hours of reperfusion</td>
<td>1.2±0.53</td>
</tr>
</tbody>
</table>

Pathologic injury only became marked (mucosal infarction) with prolonged ischaemia* in group A (30 minutes ischaemia) there was no pathologic change at the end of the ischaemia; however, there was evidence of reperfusion injury. Reperfusion injury was worse when it was preceded by longer periods of ischaemia.

Discussion

This study provides some information about the histopathologic changes that occur when the small intestine in an animal model is subjected to ischaemia and subsequent reperfusion. The findings are in keeping with findings in previous studies that the pathologic change caused by ischaemia starts in the mucosa and progresses to the deeper layers of the bowel wall to involve the submucosa and the muscle layers. During ischaemia, there was progressive worsening of pathology. The longer the length of ischaemia, the worse the ischaemia injury that was produced. In the very early stages of ischaemia, histopathologic change was not evident (group A). When short period of ischaemia (30 minutes) were followed by reperfusion, however, there was evidence of resultant injury as shown by the histologic picture of variable mucosal infarction (grade 1).

The scope of this study was limited to the evaluation of the pathological changes that were observed under the light microscope. It is a direct method of establishing tissue diagnosis. However, it is an invasive method that cannot be readily practised in the clinical setting, and there is some latency to the manifestation of the changes that are detectable by this method. The relative importance and relevance of this method, therefore, lies in its application to the investigation of other less invasive or non-invasive methods of evaluating bowel function. One such method that is under study is the use of the superconducting quantum interference device to non-invasively detect gastrointestinal electromagnetic sources. These studies show promise in the evaluation of intestinal ischaemia disease. The correlation of the histopathologic picture during mesenteric ischaemia and reperfusion will, therefore, serve as a template for evaluating intestinal viability, by comparing it with the parameters of electrical activity. For example, in group A (30 minute ischaemia) in this study, there was no evidence of pathologic change at the end of ischaemia, but there was variable mucosal infarction (grade 1) in the ensuing period of reperfusion. This is evidence of reperfusion injury. Since the injury was limited to the mucosa and did not involve the muscular layer, it can be inferred that the electrical activity emanating from the muscularis propria should be still present. It will, therefore, be important to correlate the electrical activity of the bowel during these defined period of ischaemia and reperfusion.

Histopathologic changes were generally worse during reperfusion than during ischaemia. The relationship between the effects of ischaemia is revealed further by the acutely severe changes that occurred when the preceding ischaemia lasted for a long period. In this study, there was evidence of mural necrosis (grade 4) at two to three hours of reperfusion in groups D and E. Further studies, in which the period of reperfusion is monitored for a longer time, will be required to determine if and when recovery and healing will take place, and what the histologic picture will be in those circumstances.

This study has been able to correlate the histopathologic picture with the degree of ischaemia and reperfusion injury. The effects of short duration ischaemia and subsequent reperfusion are limited to mucosal changes. Longer periods of ischaemia were attended by progressively worse but relatively slow pathologic changes, which became acutely worse and severe during reperfusion.

Acknowledgement

This study was supported by a grant from the Department of Veterans Affairs Research Service, U.S.A.

References

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