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 déficience de sang. Quelques études ont relevé une relation entre le rythme électrique normal (BER) et les changements pathologiques qui se produisent dans la cavité intestinale lors de l'injurie vasculaire. Néanmoins ces changements n'ont pas été complètement élucidés. Cette étude décrit le type histopathologique quand l'intestin grêle du cobaye était soumis à la déficience pendant un temps varié (30 – 150mn) et la reperfusion subséquent de six heures.

Les biopsies intestinales étaient portées à la baseline à la baseline à la fin de la déficience et à intervals d'une heure durant la reperfusion. L'examen microscopique des biopsies révèle l'évidence d'infarction progressive de muchose durant la déficience. Il y avait une détoriation accrue de la pathologie durant la reperfusion la séverité était plus grande que quand la reperfusion était précedée par une plus longue période de déficience. Ces changement étaient significants statistiquement. Le model observé dans cette montre clairement que l'injurie de reperfusion est reflecteé dans la réponse histopathologique et que ça s'empire en séverité que dans la réponse à la déficience. Les études d'une durée plus longue peuvent clarifier l'image durant le rétablissement de l'injurie de la déficience reperfusion de la cavité 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 [1,2,3,4]. The pathologic changes in the bowel during ischaemia have also been correlated to the change in electrical activity of the intestine[5]. The pathologic changes range from mucosal infarction in early ischaemia to mural necrosis after prolonged ischaemia,[7]. 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[8]. 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[1,2,5]. Biomagnetic fields that are related to the electric 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[9]. 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[4,10]. Acute mesenteric ischaemia is attended by high morbidity and mortality rates[11], 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 anaesthetised. 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 manouevres, using a Doppler flow probe (Koven Technology, Inc., model ES-1000SPM). Biopsy of the gut segment was done

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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 ballon 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 ballon 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 euthanised 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 Swerdlow and Antonioli system[12], 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 of ischaemia) there was a linear progression ($R^2 = 0.89$) 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.

Table 1 Histopathologic scoring based on a modification of the Swerdlow and Antonioli system[12]

Grade	Histology
0	No pathologic change
1	Focal loss of surface epithelium
2	Mucosal infarction • extensive loss of surface epithelium • loss of variable amounts of lamina propria • sparing of basal glands • intact muscularis mucosae
3	Submucosal infarction •variable necrosis of submucosa • completee mucosal necrosis • intact muscuularis mucosae
4	Mural infarction • loss of muscularis mucosae • complete necrosis of mucosa and submucosa
5	Mural infarction • involvement of inner layer of muscularis propria • complete necrosis of mucosa and submucosa
6	Transmural necrosis of entire bowel wall.

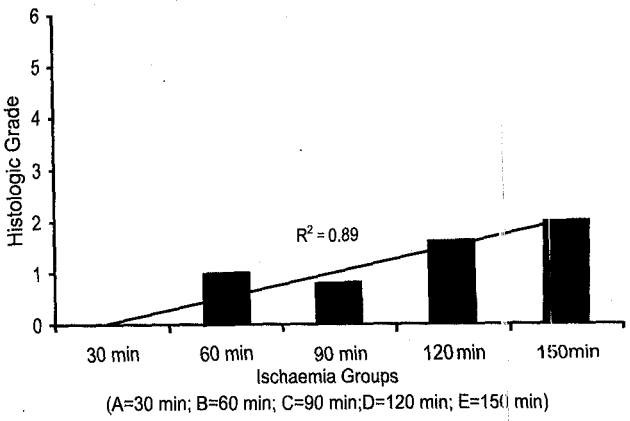

Fig. 1 *Histologic grades of all experimental group at the end of ischaemia. The groups were subjected to ischaemia for different period of ischaemia: A = 30; B = 60, C = 90, D = 120, E = 150 minutes. There was a linear progression in the pathologic changes ($R^2 = 0.89$). The histologic grades for B, C, D and E were not statistically different. min = minutes.*

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

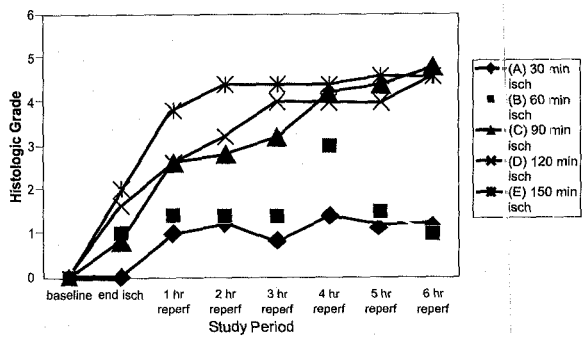

Fig. 2 *Mean histologic grades in each group (A,B,C,D,E) plotted against time of ischaemia and reperfusion. There was no observed pathologic change at the end of ischaemia in group A. Following ischaemia of long periods in groups C, D, and E, the acute worsening of pathology during reperfusion (grade 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*

Table 2 Histopathology of the bowel at the end of ischaemia of varying periods and at different time points during perfusion.

	Mean histologic grades ± SEM				
Group	**A**	**B**	**C**	**D**	**E**
Length of Ischaemia	30mins	60 mins	90 mins	120 mins	150 mins
At Baseline	0	0	0	0	0
At end of Ischaemia	0#	1±0.33	0.8±0.06	1.6±0.8	2±0*
After 1 hour of reperfusion	1±0.33ƒ	1.4±0.73	2.6±1.2	2.6±0.6	3.8±0.67j
After 6 hours of reperfusion	1.2±0.53ƒ	1±0.33	4.8±0.3	4.6±0.34	4.6±0.4j

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 injuryƒ. Reperfusion injury was worse when it was preceeded by longer periods of ischaemia.j*

pathologic picture was that of mucosal and submucosal infarction (grade 4). The score remained at grade 4 until the end of six hours of reperfusion. In other words, the pathologic grade was worse when reperfusion was preceded by longer periods of ischaemia. All groups showed some degree of mucosal infarction or submucosal necrosis at the end of one hour reperfusion, including group A (30 -minute ischaemia), which was the only group that did not show any pathologic change at the end of ischaemia. During the period of the experiment, no group showed any return to baseline values (grade 0).

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[7] 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 worsen 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[4,9]. These studies show promise in the evaluation of intestinal ischaemic disease[4,13]. The correlation of the histopathologic picture during mesenteric ischaemia and reperfusion will, therefore, serve as a template for evaluating instestinal 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.

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References

1. Schamaun M. Electromyography to determine viability of injured small bowel segments; an experiemental study with

preliminary observations. Surgery 1967; 62: 899 – 909.

2. Szurszewski J, Steggerda FR. The effect of hypoxia on the electrical slow wave of the canine small intestine. Am. J. Dig. Dis 1968; 13: 168 – 177.

3. Cabot R, Kohatsu S. The effects of ischaemia on the electrical and contractile activities of the canine small intestines. Am. J. Surg. 1976; 136: 242 –246.

4. Richards WO, Garrard CL, Allos SH, Bradshaw LA, Staton DJ, Wikswo J. P Jr. Noninvasive diagnosis of mesenteric ischaemia using a SQUID magnetometer. Ann. Surg. 1995; 221(6): 696 – 705.

5. Garrard CL, Halter S, Richards WO. Correlation between pathology and electrical activity during acute intestinal ischaemia. Surg Forum 1994; 45: 368 – 371.

6. Amano H, Bulkley G, Gorey T. The role of microvascular patency in the recovery of the small intestine from ischaemic injury. Surg Forum 1980.

7. Mitsudo S, Brandt L Pathology of intestinal ischaemia. In: Intestinal ischaemia. (Surg. Clin North Am) eds. Boley S, Brandt L. Philadelphia: W.B Saunders. 1992; 72(1): 43 – 660.

8. Hedge SH, Seidel DA, Ladipo JK, Bradshaw LA, Halter S, Richards WO. Effects of Mesenteric ischaemia and reperfusion on small bowel electrical activcity. J Surg. Res 74(1): 86 – 95.

9. Bradshaw L, Allos SH, Wikswo JP Jr, Richards WO. Correlation and comparison of magnetic and electric detection of small intestinal electrical acticvity. Am J. Phiol. 1997; 272: G1159 – 1167.

10. Golzarian K, Staton DJ, Wikswo JP Jr, Friedman RN, Richards WO. Diagnosing intestinal ischaemia using a noncontact superconducting Quantum Interfernece Device Am J Surg. 167: 586 – 591.

11. Williams L. Mesenteric ischaemia. In: The Acute Abdomen. eds. J. Sawyers and L. Williams L. Mesenteric ischaemia. In: The Acute Abdomen. eds. J. Sawyers and L. Williams, Philadelphia, PA: Saunders, 1988; 331 – 353.

12. Plonka AJ, Schentag JJ, Messinger S, Adelman MH, Francis KL, Williams JS. Effects of enteral and intravenous antimicrobial treatment on survival following intestinal ischaemia in rats. J Surg Res 1989; 46: 216 – 220.

13. Allos SH, Bradshaw LA, Wikswo JP Jr, Richards WO. The use of the SQUID magnetometer for the diagnosis of ischemia caused by mesenteric venous thrombosis. World J Surg 1997; 21: 173 – 178.