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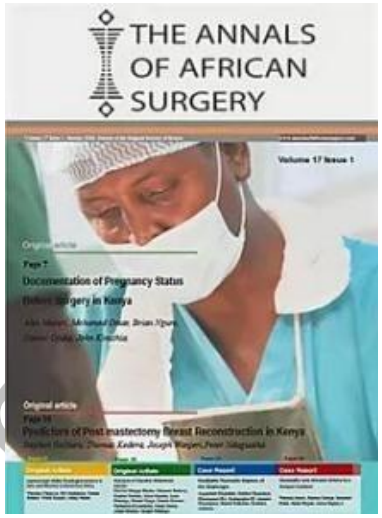

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Influence of Intestinal Strangulation Release on Ischemia-Reperfusion Injury in Sprague-Dawley Rat

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Abstract

Background: In intestinal ischemia, reperfusion towards the injured intestine can cause further injury to the intestine itself and remote organs. This research aims to evaluate the influence of intestinal strangulation release before resection on the intestine outside margin of the strangulated intestine, compared with subjects without intestinal strangulation release.

Methods: Fourteen male Sprague-Dawley rats were subject to strangulation of one loop distal ileum for four hours. On the strangulation release (SR) group, the strangulated intestine was released for 5 minutes and then resected for necrotic parts. On the without strangulation release (WSR) group, the strangulated intestine was immediately resected without strangulation release. The control group received sham laparotomy. Four hours after the second laparotomy, the animals were sacrificed, and intestinal samples were taken for histo-morphological analysis and measurement of intestinal malondialdehyde (MDA) level.

Results: The histo-morphological intestinal mucosal injury and intestinal MDA level are insignificantly higher on the SR group compared to the WSR group ($p>0.05$).

Conclusion: Intestinal strangulation release before resection causes more tissue injury and oxidative stress on the intestine outside the strangulation part, but the difference is not statistically significant

Keywords: Ischemia-reperfusion injury, intestinal ischemia, intestinal strangulation release, malondialdehyde, intestine injury

Introduction

Intestinal strangulation is one of the most common surgical cases that can interfere with the flow of blood vessels to the intestine as in the case of hernias, volvulus, and intussusception, which also causes half of all death case in small intestine obstruction (1,2). Intestinal strangulation along with its complications has a morbidity rate of 40% (3). Ischemia-reperfusion (I / R) injuries can affect many organs, but the intestine is one of the most susceptible organs to this condition (4). Disorders of blood flow to the intestine can cause ischemic injury, which causes tissue injury, but more complicated condition can happen when the previously ischemic tissue was restored its blood flow to maintain cell function, known as reperfusion injury. Injuries due to reperfusion often outweigh injuries caused by the previous ischemia (1,4,5).

When the ischemic condition occurs, hypoxanthine as the by-product of adenosine triphosphate was accumulated. Then when tissue reperfusion occurs, hypoxanthine and oxygen produce superoxide which causes inflammatory responses and tissue injury (1,6). In intestinal ischemia with clear boundaries, a release is often performed to assess doubtful intestinal viability. However, it should be considered that the act of strangulation release can cause reperfusion injury, which may be prevented by resection of all the strangulated part without releasing the strangulation. Knowledge about the effects of intestinal strangulation release is expected to be a consideration for deciding whether strangulation release should be carried out before resection of the ischemic intestine. The best parameters for assessment of intestinal injury without clinical signs are histopathological examination and malondialdehyde (MDA) levels.

This research aims to determine differences in intestinal histo-morphological changes and intestinal MDA level in locations adjacent to strangulation, between intestines that are resected with and without previous strangulation release.

Material and Methods

Animals

This research was experimental with male Sprague-Dawley rats. The subject that selected were male sex, healthy, age 8 - 12 weeks age with a median bodyweight of 183.50 (174-213) grams. Subjects were received and raised in *Pusat Penelitian dan Pengembangan Kesehatan (Puslitbang)*, Health Research and Development Agency, Ministry of Health of the Republic of Indonesia. This research was approved by the Ethics Committee *Penelitian Kesehatan Fakultas Kedokteran Universitas Indonesia* No. 0554/UN2.F1/ETIK/2018 and follow animal ethics. The sample size in this research was calculated using the Federer formula approach and considered a 10% drop out correction.

The subjects were given ad libitum food and drinks before treatments, assessed the general condition, and weighed to get the same sample. Animals were raised in plastic cages sized 50 x 35 x 15 cm with a battery system base. Cages were placed at room temperature, and light uses indirect sunlight in the same cycle as nature.

Experimental Procedures

This research included 14 subjects, consisted of 2 rats in the control group, 6 rats in the group that received resection after strangulation release (SR), and 6 rats in the group that received resection without strangulation release (WSR).

At the time of the experiment, subjects were anesthetized with 50 mg/kg BW dose of ketamine and 7 mg/kg BW dose of xylazine intramuscular injection.

A median laparotomy incision of 3 cm was performed along the abdomen. On two treatment groups, a 10 cm length loop of distal ileum was strangulated using 2.0 silk ligation, and then the abdomen was closed.

Distal ileum was chosen in this study due to the consideration as one of the commonest location of

strangulations, especially caused by intussusception cases (7). Specifically, in small intestine, reperfusion after ischemia initiates systemic inflammatory responses which lead to cell and tissue injuries (8). Hence, I/R injury in the small intestine is considered as an important clinical problem leading to high mortality and morbidity. The small intestine total length observed on a subject in this study was 100 cm. This was approximated from a previous study which stated that a 125 gram of rat had a 95 cm in small intestine length and would lengthens 5 cm approximately for each 100-gram body weight increment (9). Hence, the ratio of strangulated gut vs total gut length in this study was 1: 10.

Ligation was released after four hours of strangulation. Reperfusion duration was determined to be 15 minutes. This reperfusion time was chosen to resemble the clinical setting realty where 15 minutes is the maximum time allocated to evaluate the intestine viability. Then, resection of the necrotic intestine was performed after evaluate the intestinal viability on the SR group, then the abdomen was closed. Meanwhile, in the WSR group, resection of all ischemic intestine was performed without releasing the ligation.

Four hours later, two intestinal samples from each rat were taken through a surgical procedure. All of the samples I was fixed in 10% formalin buffer for histomorphological examination. All of the samples II were put into containers that contained 0.9% NaCl and stored in 20°C storages for tissue MDA level measurement. The subjects were then sacrificed by extracting all of the blood directly from the hearts. MDA is used as an indicator because MDA is the final product of oxidative decomposition which is initiated by radicals from polyunsaturated fatty acids (10,11).

Histopathological examination of the intestine

After the paraffin block was made from the sample I, it was cut 4 ɥm thick, and hematoxylin and eosin staining was performed. All histomorphological examinations were observed by a pathologist blindly. Mucosal lesions were classified by a scale of 0 to 5, as described by Chiu et al (12) (Table 1).

Malondialdehyde Examination of Intestinal Tissue

Thiobarbituric Acid (TBA) reaction to produce MDA − TBA adduct. MDA measurement results were listed in nmol/mL using MDA Assay Kit.

Statistical Analysis

The degree of intestinal mucosal injury and intestinal MDA levels were presented in numerical data. A numerical data normality test was performed using the Shapiro − Wilk test. Kruskal-Wallis test was used for abnormal distribution numerical data. One Way Anova test was used for normal distribution numerical data. Statistically significant differences were obtained if $p < 0.05$.

Results

Fourteen male, healthy Sprague-Dawley Rats were divided into 3 groups. No significantly different body weight and tissue sample weight were found between control, SR group, and WSR group. Complete data of weight and tissue weight between control and treatment groups are shown in *Table 2*.

A comparison of histomorphological intestinal mucosal injury grade of each group was shown in Table 3. In the analysis, there were insignificant differences in intestinal mucosal injury grade between the control group, SR group, and WSR group. Representatives of intestinal mucosal injury histological images are provided in *Figure 1*.

In the analysis of MDA levels in the control group, DR and R treatments, using the One-way Anova parametric test for intestinal MDA, MDA levels between groups showed a result of $p > 0.05$. The complete data can be seen in *Table 3*.

Discussion

In this research, it was found that there was an increase in intestinal injury in the SR group but with an insignificant difference. The model of strangulation in this study was made from a 10 cm length loop of distal ileum. This ileal loop was considered potential to induce a partial critical intestinal ischemia, compared to occlusion of cranial mesenteric artery (CMA) as a global critical intestinal ischemia (13). Moreover, this insignificant result might be due to the small sample size as a weakness of this study. A previous research by Yamamoto et al (14) showed macroscopic findings of the intestine of Wistar rat that undergoing reperfusion after superior mesenteric artery clamp model. It was found that the intestine turned pale during the ischemic period, but worsens after reperfusion that the intestine became cyanotic along with parenchymal haemorrhage. As for the microscopic findings of the intestine, it showed mucosal injury including denudation of mucosa and neutrophil infiltration to the injured mucosal layer that happens during ischemia period but exacerbated after reperfusion.

Another study by Guan et al. using remote controlled inflatable occluder of mouse jejunum and peri-intestinal marginal artery occluder, as long-term ischemia, resulted in irreversible epithelial cell structure and function (15). Moreover, research by Parks and Granger showed that mucosal injuries that occur after 3 hours of ischemia followed by 1 hour of reperfusion, greater than mucosal injuries produced after 4 hours of ischemia, without reperfusion (16). Research by Caty et al using rats Sprague-Dawley as a model of intestinal I / R, showed increased levels of histamine, xanthine oxidase, and xanthine dehydrogenase in ischemia 120 minutes and reperfusion for 15 minutes. The xanthine oxidase activity which measured after 15 minutes of reperfusion has increased as much as 140% than xanthine oxidase activity of 120 minutes of ischemia alone (17). These results indicate that intestinal mucosal injury is more severe due to the action of reperfusion of the intestine itself rather than during the ischemic period (16).

Ischemia-reperfusion injury not only injured the local tissue but also causes a systemic inflammatory response. A study by Grosche et al. reported activation of eosinophils, other leukocytes, and nitrogen radicals production after ischemia and reperfusion in the colon of horses. These cells potentially involved in the inflammatory response. Moreover, they also reported apoptosis in the large colonic mucosa itself which might be the prominent cause of cell death during ischemia and reperfusion (18). Intestinal mucosal injury of ischemia-reperfusion results in failure to maintain a mucosal barrier that leads to increased plasma endotoxin level which indicates bacterial translocation (16). The ischemia-reperfusion injury also known as one of the main causes of multiple organ failure (19,20). Therefore, in cases of intestinal strangulation, where the segment of the intestine in question seems to be non-vital, then the act of resection of the intestine without strangulation release or reperfusion is first expected to prevent reperfusion injuries that occur.

The intestine consists of unstable enterocytes which can easily get injured due to ischemia, this also makes the intestine most susceptible to I / R injury (5,18). Enterocytes located at the tip of the microvilli are more sensitive to the effects of ischemia than enterocytes that located on the crypt. This is due to its location at the end of the distribution of central arterioles and relatively less collateral blood flow so that the PaO in the distal enterocytes is lower than in the crypt. Xanthine oxidase activity, which is the main source of free radicals, is found mainly in the intestinal mucosal lining with increased activity from the bottom of the villus to the tip. This is also one of the reasons for the increased sensitivity of the end of the villus to I / R injuries compared to the bottom of the villus (21).

This research also showed an insignificant difference in intestinal MDA levels in intestine tissue outside the strangulation margin between all groups. This also might be due to the model of

strangulation and time of reperfusion differences. However, previous studies with different models showed significant results. Research by Akman et al using Wistar rat showed that the intussusception model by inserting ileum with stylets for 4 hours resulted in a significant increase in MDA levels (8). While Zheng et al in his research obtained intestinal MDA values of 1.01 ± 0.11 for rats Sprague-Dawley control and MDA value of 2.30 ± 0.19 in rats treated with clamps in the superior mesenteric artery for 45 minutes and reperfusion for 120 minutes (22). Nilsson et al (23) showed that I/R injury occurs after 2- 5 minutes of intestinal reperfusion that cause oxidant production immediately on the ischemic intestine. The oxidant production after reperfusion originally comes from the electron transport chain in mitochondria, xanthine oxidase, metabolism, endothelial cells, prostaglandins, and activated neutrophils (20).

Conclusion

This research shows that intestinal strangulation release before resection causes more tissue injury and oxidative stress on the intestine outside the strangulation margin but insignificant difference. Hence, an intestinal resection without any release procedure is recommended on the strangulated intestine with a low chance of viability.

Conflict of Interest

The authors declare that there is no conflict of interest in this research.

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References

1. Gonzalez LM, Moeser AJ, Blikslager AT. Animal models of ischemia reperfusion-induced intestinal injury: progress and promise for translational research. Am J Physiol Gastrointest Liver Physiol. 2015; 308: G63-75.
2. Hayanga AJ, Bass-Wilkins K, Bulkley GB. Current management of small-bowel obstruction. Adv Surg. 2005; 39: 1–33.
3. Shatila AH, Chamberlain BE, Webb WR. Current status of diagnosis and management of strangulation obstruction of the small bowel. Am J Surg. 1976; 132(3): 299–303.
4. Teke Z, Sacar M, Yenisey C, et al. Activated protein C attenuates intestinal mucosal injury after mesenteric ischemia/reperfusion. J Surg Res. 2008; 149(2): 219-30.
5. Mallick IH, Yang WX, Winslet MC, et al. Ischemia-reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci. 2004; 49(9): 1359-77.
6. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology. 2001; 94: 1133-38.
7. Lissauer T. Pediatric emergencies: a practical guide to acute paediatrics. Springer Netherlands; 2012. p. 103.
8. Watanabe T, Tanigawa T, Kobata A, et al. Toll-like receptor 2 mediates ischemia-reperfusion injury of the small intestine in adult mice. PLoS One. 2014; 9(10): 1–7.
9. Miller DL. Rat small intestine: development, composition and effects of perfusion. Am J Dig Dis. 1971; 16(3): 247-54.
10. Akman H, Somuncu S, Dikmen G, et al. Protective effect of selenium on intussusception-induced ischemia/reperfusion intestinal oxidative injury in rats. Turk J Med Sci. 2010; 40(3): 391-97.
11. Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev. 2014; 2014: 360438.

12. Chiu CJ, McArdle AH, Brown R, et al. Intestinal mucosal lesions in low-flow states. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg. 1970; 101(4): 478.

13. Kozhura VL, Basarab DA, Timkina MI, et al. Reperfusion injury after critical intestinal ischemia and its correction with perfluorochemical emulsion "perftoran." World J Gastroenterol. 2005; 11(45): 7084–90.

14. Yamamoto S, Tanabe M, Wakabayashi G, et al. The role of tumor necrosis factor-α and interleukin-1β in ischemia-reperfusion injury of the rat small intestine. J Surg Res. 2001; 99(1): 134–41.

15. Guan Y, Worrell RT, Pritts TA, et al. Intestinal ischemia-reperfusion injury: Reversible and irreversible damage imaged in vivo. Am J Physiol Gastrointest Liver Physiol. 2009; 297(1): G187–96.

16. Parks DA, Granger DN. Contribution of ischemia and reperfusion to mucosal lesion formation. Am J Physiol. 1986; 250(6): G749-53.

17. Caty MG, Schmeling DJ, Friedl HP, et al. Histamine: a promoter of xanthine oxidase activity in intestinal ischemia/reperfusion. J Ped Surg. 1990; 25(2): 218-23.

18. Grosche A, Vet M, Freeman DE, et al. on Production of Nitrotyrosine, Activation of Mucosa of Horses. Am J Vet Res. 2012; 73(1): 53–61.

19. Watson MJ, Ke B, Shen XD, et al. Intestinal Ischemia/Reperfusion Injury Triggers Activation of Innate Toll-Like Receptor 4 and Adaptive Chemokine Programs. Transplant Proc. 2008; 40(10), 3339–41.

20. Cerqueira NF, Hussni CA, Yoshida WB. Pathophysiology of mesenteric ischemia / reperfusion: a review. Acta Cir Bras. 2005; 20(4): 336-43

21. Sasaki M, Joh T. Oxidative stress in digestive disease. J Clin Biochem Nutr. 2007; 40(1): 1-12.

22. Zheng XF, Mao YF, Cai JM, et al. Hydrogen-rich saline protects against intestinal ischemia/reperfusion injury in rats. Free Radic Res. 2009; 43(5): 1-7.

23. Nilsson UA, Lundgren O, Haglind E, et al. Radical production during in vivo intestinal ischemia and reperfusion in the cat. Am J Physiol. 1989; 257: G409-14.

Table 1: Histopathological grading criteria for intestinal mucosal injury

Grade	Histopathological findings
0	Normal mucosal villi.
1	Development of subepithelial Gruenhagen's space, usually at the apex of the villus; often with capillary congestion.
2	Extension of the subepithelial space with the moderate lifting of epithelial layer from the lamina propria.
3	Massive epithelial lifting down the sides of the villi. A few tips may be denuded.
4	Denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted.
5	Digestion and disintegration of lamina propria; hemorrhage and ulceration.

Table 2: Comparison of body weight and tissue weight between the control group, the strangulation release group, and the without strangulation release group

Variable	Control	SR	WSR	p-value
Body weight (gram)	186.50 (185-188)	184.00 (176-213)	182.00 (174-193)	0.789*
Small intestine tissue weight (mg)	103.45±2.05	104.83±4.47	102.45±1.22	0.456**

*Kruskal-Wallis test **One-way Anova test

SR: Strangulation release

WSR: Without strangulation release

Table 3: Comparison of intestinal mucosal injury scoring and intestinal MDA levels in the control group, the strangulation release (SR) group and the without strangulation release (WSR) group

	Control	SR	WSR	p-value
Scoring of Intestinal Mucosal Injury	**0.5 (0-1)**	**1.5 (0-3)**	**0.5 (0-1)**	**0.078**
Intestinal MDA level (nmol/mL)	**1.15±0.41**	**1.30±0.43**	**1.26±0.46**	**0.915***

*one-way Anova

MDA: malondialdehyde

SR: Strangulation release

WSR: Without strangulation release

Figure 1: Intestinal mucosal injury histological images