Novel and simple method using cable ties to induce intestinal strangulation in a rat model

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ABSTRACT

BACKGROUND The pathogenesis of intestinal fibrosis after strangulation in abdominal surgery is not fully understood. Developing an experimental and reliable method is needed to overcome this problem. This study aimed to develop an effective method for intestinal strangulation leading to fibrosis, which might induce intestinal obstruction.

METHODS This study was conducted from 2018 to 2019 at the Department of Pharmacology, Faculty of Medicine, Universitas Indonesia. A total of 24 Sprague Dawley rats were used in this study. Artificial intestinal strangulation using cable ties made of durable plastic, which is generally used to ligate objects, was applied, and rats were further classified into control, 6-hour, and 24-hour strangulation groups. At the end of the study, the rats were sacrificed, intestinal tissues were collected, and histomorphological changes were observed using hematoxylin and eosin stain. Moreover, Masson’s trichrome staining was used to assess collagen density.

RESULTS Median collagen density score of the 24-hour strangulation group was higher than the control. A significant difference in collagen density was found between the submucosal layer of the 24-hour strangulation group and the control (45.4 [11.4] versus 32.4 [14.0], \(p<0.001\)) and between the mucosa of the 6- and 24-hour strangulation groups and the control (26.9 [17] versus 6.46 [4.3], \(p=0.01\) and 24.9 [8] versus 6.46 [4.3], \(p=0.004\), respectively).

CONCLUSIONS The simple use of cable ties adequately promotes intestinal strangulation.

KEYWORDS collagen, fibrosis, intestinal obstruction
resorbed. Excessive deposition compared to resorption may lead to the development of fibrosis. Healing intestinal ischemia associated with an inflammatory response leads to fibrosis. This study aimed to determine the intestinal strangulation method in a rat model using plastic cable ties. Strangulation is denoted based on collagen deposition and fibrosis observed in situ.

METHODS

This study was carried out at the Faculty of Medicine, Universitas Indonesia in February 2018 using 24 Sprague Dawley rats of 6–8 weeks and weighed 150–200 g. The Ethics Committee of the Faculty of Medicine, Universitas Indonesia approved this study (No: 0261/UN2.F1/ETIK/2018). The sample was calculated using the Federer method regarding the concept of reduction, replacement, and refinement. The rats were randomly divided into three groups, namely the control, 6-hour, and 24-hour strangulation groups. The control group had laparotomy using a simple strangulation to the ileum after an immediate release (Sham surgery). Female, unhealthy, and dead rats were excluded from the criteria. In the 6- and 24-hour strangulation groups, intestinal strangulation was proceeded to the ileum, which represents the most herniated intestines in humans. The ileum was subjected to strangulation using plastic cable ties.

Strangulation method

The procedures were carried out through laparotomy surgery under ketamine hydrochloride 80 mg/kg body weight (BW) with xylazine 10 mg/kg BW intraperitoneal. Three ties were placed in sequence with 1 cm distance; over an encircled standard plastic sheet covering the intestines for protection purposes, ties were tightened to 4 mm in diameter (Figure 1).

Nylon ties were chosen due to their properties, flexibility, and several teeth features to guide the measurements, which can sufficiently investigate tissue strangulation. A cable tie consisted of 118 teeth along the cable. Nine teeth were required to produce 4 mm in diameter ties, leaving 109 teeth, which were counted as measurement guidance. After surgery, the rats were kept in a warmed blanket during recovery. The rats were re-operated to release strangulation at 6 and 24 hours after cable ties were applied. Six hours were set before the ischemic time limit became irreversible (maximum of 8 hours), while 24 hours were set to limit the necrosis found in ischemic tissues. The rats were then sacrificed following the regulation of the animal lab.

Histomorphological, collagen density, and fibrosis assessment

The specimens were prepared for histomorphological study to assess the tissue damage using hematoxylin and eosin stain and the collagen density in samples stained using a Masson’s trichrome technique. Each layer of the specimen was carefully observed to differentiate each histomorphological change. The collagen density was evaluated using ImageJ software (Wayne Rasband, USA) on the samples investigated under the light microscope. The histomorphological change was assessed using Park/Chiu method, in which tissue damage was scored from 0 to 8. The tissue condition was described as follows: normal (score 0), the presence of subepithelial space...
in villi ends (score 1), extended subepithelial space with moderate epithelial lifting (score 2), subepithelial space with partial denuded mucous lining (score 3), denuded epithelial lining with capillary dilatation (score 4), and disintegrated lamina propria (score 5). Moreover, Park et al.¹⁷,¹⁸ expanded the scoring as follows: damaged crypt (score 6), transmucosal infarction (score 7), and transmural infarct (score 8). A higher score represented the worse disruption.

The fibrosis of the intestine was assessed using the Theiss’s scoring: score 0 showed no increased collagen deposition, score 1 showed increased collagen deposition in the submucosa, score 2 showed increased collagen deposition in submucosa and mucosa, score 3 showed increased collagen deposition in muscularis mucosa, submucosa, and mucosa, and thickening disorganization of the muscularis mucosa, score 4 showed increased collagen deposition in muscularis propria, muscularis mucosa, submucosa, and mucosa, and score 5 showed increased collagen deposition throughout all layers, including serosa.²⁰⁻²¹ The collagen density represented by section involvement as a percentage: score 1 showed 0–25% of the section, score 2 showed 25–50% of the section, score 3 showed 50–75% of the section, and score 4 showed 75–100% of the section.²⁰ Data were further analyzed using a comparative analytical study proceeded using Mann–Whitney analytical test by SPSS software version 20.0 (IBM Corp., USA).

**RESULTS**

The histomorphological change observed using a light microscope showed tissue disruption, with the lowest score of 0 in the control and the highest score of 3 in the 24-hour strangulation group. Collagen deposition was observed with the lowest score of 4 in both the 6- and 24-hour strangulation groups; the highest score of 5 was observed in the 24-hour strangulation group (Figure 2a). Significant collagen deposits were found in the submucous layer of the 24-hour strangulation group (Figure 2b).

In the serosa, no difference in collagen density was found between the two groups. Meanwhile, there

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**Figure 2.** Intestinal collagen deposition. (a) Microscopic examination of collagen deposit in submucous layer at 6 hours after strangulation (arrow); (b) collagen deposit between the two groups; (c) fibrosis score between the two groups; (d) mean collagen percentage distribution. SD=standard deviation. *Mann–Whitney post-hoc analysis; †Mann–Whitney test; ‡one-way ANOVA; §post-hoc Bonferroni analysis
was a significant difference of collagen deposit in the muscularis layer between the 24-hour strangulation group and the control (p = 0.001) and in the muscularis mucosa between the 6- and 24-hour strangulation groups and the control (p = 0.011 and p = 0.026, respectively) (Figure 2c). The 24-hour strangulation group had a higher collagen density median score than the control. A significant difference in collagen density was found between the submucosal layer of the 24-hour strangulation group and the control (45.4 [11] versus 32.4 [14.0], p<0.001) and between the mucosa of the 6- and 24-hour strangulation groups and the control (26.9 [17] versus 6.46 [4.3], p = 0.01 and 24.9 [8] versus 6.46 [4.3], p = 0.004, respectively). A significant difference was found between the 6- and 24-hour strangulation groups and the control (p<0.05 and p<0.01, respectively) (Figure 2d).

**DISCUSSION**

Inducing intestinal obstruction in an animal model is quite challenging. Although the pathogenesis is clear, no effective method was available to induce fibrosis that may lead to intestinal obstruction. A study used different methods to provide intestinal obstruction, namely penetrated braided silk, half ligation method, and using wide (6 mm) and narrow (2 mm) tubes surrounded the intestine.¹⁷ The wide tube group showed a significant difference in macroscopic and histological features, intestinal transit ratio, contractility, circumference, and wet weight compared with the other groups. Another study used a silicone ring in the distal intestines to induce a partial intestinal obstruction.²² Following the unsuccessful tries of these proposed methods in our preliminary study (not reported here), we used cable ties to induce strangulation experimentally. The availability and qualities of cable ties, which allow for several measuring teeth along the cable, aid in measurement guidance. The ties were applied to encircle the intestinal diameter and tighten it as if the actual hernia ring was pinched. The cable tie should be wide enough to pinch the segment. Thus, a plastic tube was placed before the cable tie.

The histomorphological features showed pathologic changes along the strangulated segment, represented by a higher Chiu/Park score in the 24-hour strangulation group. In the present study, the collagen deposit increased with the strangulation period. A higher collagen density in the acute setting led to the increase of extracellular matrix, which might increase the possibility of fibrosis. The deposits distributed in this study showed a higher collagen density in the submucosa of the strangulated segment. This finding is in line with the previous study that showed a higher proliferation of fibroblasts in the intestine submucosal layer.¹⁸

Fibrosis is a logical consequence of increased collagen density. A study showed that the myofibroblast activation in inflammatory response led to excessive deposition of the extracellular matrix.²³ The extracellular matrix component comprises the structure that directly regulates the inflammatory response and fibroproliferation through focal adhesion with both immune and non-immune cells, particularly myofibroblasts.⁴ Thus, an excessive collagen deposit as an extracellular matrix constituent may lead to intestinal fibrosis, stricture, and obstruction.⁴

Another valuable finding in the present study was the strangulation duration, which was set at 6 and 24 hours. A 6-hour period in rodents implies 6 days in humans, while 24 hours in rodents is equal to 20–21 days in humans.¹⁴ This setting clinically implies the inflammatory phases; and the longer the inflammatory phase, the greater the fibrosis is found. This study’s limitation was no accurate measures of the strangulation that reflected the obstruction of blood flow. Besides, oxygen saturation to ensure hypoxia was not measured.

Fibrosis is characterized by collagen deposition following an inflammatory response to an event such as trauma, infection, and ischemia. The fibrosis may manifest within a few days to a week after the event. However, in daily practice, fibrosis manifested in intestinal obstruction may be found within 5 days to 22 years after the release of a strangulated hernia.¹ Fibrosis is a logical consequence. This finding implies that simple plastic cable ties can cause intestinal strangulation. In conclusion, the simple use of cable ties adequately simulates intestinal strangulation leading to a higher collagen deposit denoting fibrosis after 24 hours of treatment.

**Conflict of Interest**

The authors affirm no conflict of interest in this study.
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