Comparison of Colonic Anastomosis Using Dry Amnion Membrane and Fibrin Glue in Intraperitoneal Infection Condition Assessed from Tissue Hydroxyproline Level Measurement (Study on Wistar Rat)

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Abstract

Background : Anastomotic leak in the colon is one of the causes of high morbidity and mortality in infectious cases. The morbidity and mortality rates in patients with colonic anastomotic leakage range from 7–39%. Various efforts have been made to prevent leakage, such as changing suture techniques and using additional materials. Materials such as fibrin glue and amniotic membranes are gaining popularity in the wound healing process.

Purpose : To compare the use of dry amniotic membrane and fibrin glue in colonic anastomosis in intraperitoneal infection assessed with tissue hydroxyproline level measurement.

Method : This study is an experimental. The subjects were divided into 3 groups, group I (anastomosis using a simple interrupted suture only), group II (anastomosis and was applied with dry amniotic membrane) and group III (anastomosis using a simple interrupted sutureand was applied with fibrin glue). The anastomotic segment was taken and made into homogenate, then measured for the hydroxyproline level.

Result : The study was conducted on 27 Wistar rats, male rats that have been aged 10-12 weeks with the weight of 250-300 grams. It was obtained the differences in average levels hydroxyproline in the group I = 2157.41 (± SD = 478.60), group II = 2887.40 (± SD = 688.49) and group III = 2224.59 (± SD = 416.63). ANOVA test at hydroxyproline samples showed that P <0.05 (0.015) means that there are statistically significant differences in the levels of hydroxyproline in all three groups of samples. Post Hoc test of ANOVA showed that the levels of hydroxyproline in the group II (Interrupted suture + Dry amniotic membrane) has the most high mean difference with the significance of p<0.05 means that the levels of hydroxyproline in the group is most significant.

Conclusion : Based on the results of the study, it can be concluded that the use of the dry amniotic membrane was better than fibrin glue of colonic anastomosis in conditions of intra-peritoneal infection, there was an increase in hydroxyproline levels of colonic anastomosis tissue applied in dry amniotic membrane with statistically significant results.

Keywords *: colonic anastomosis, peritonitis, fibrin glue, dry amniotic membrane, collagen, hydroxyproline*

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Background

Anastomotic leak in the colon is one of the causes of high morbidity and mortality in infectious cases. The morbidity and mortality rates in patients with colonic anastomotic leakage range from 7-39%. In the

case of colonic anastomotic resection accompanied by peritonitis, the leakage rate was increased by 2.5 times[1]. The prevalence of anastomotic leak in the right colon was lower (1.35%) than the left colon (5.20%) than all cases of anastomotic leak in the colon[2,3]. A leaky colonic anastomosis will cause the peritoneal cavity to be contaminated by bacteria, causing peritonitis. One of the causes of leakage of a colonic anastomosis in patients with peritonitis is bacterial contamination of the sutures.

The working principle of fibrin glue in closing anastomotic suture wounds is by acting as a barrier to the wound area (sealant), where the fibrin glue will freeze within ± 3 seconds to form a sealant, so bacteria cannot enter the anastomose (antibacterial) suture. There is no external factor in this case, namely intraperitoneal bacteria which can serve ordering fibroblasts and collagen, by presenting fibrin glue it is hoped that the healing process involving ordering fibroblasts and collagen runs normally. It is quite expensive to use fibrin glue[4]. In the study by Senol, et al., Giving fibrin glue to a group of rats with peritonitis obtained significant results where in the peritonitis group treated with fibrin glue, the yield density of collagen was higher than that in the group not given fibrin glue[5]. However, in a study conducted by Nordentoft, et al., Giving fibrin glue did not have a significant effect or benefit on the anastomosis process in the digestive tract[6]. The amniotic membrane is a material that is widely used to help stimulate the healing process[7].

The amniotic membrane contains components of growth factor and proteinase inhibitors[8]. From research, it was found that the amniotic membrane has antibacterial properties, can help the process of epithelialization and wound healing, inhibits inflammation and scar ordering, and increases angiogenesis[7]. Membrane amnions have another feature of containing large amounts of growth factors, cytokines, and extracellular matrix, which play an important role in wound healing, and serve as a base or scaffold for new tissue growth and tissue engineering[9].

In a study conducted by Uludag, et al., The use of amniotic membranes in colonic anastomosis provides significant effects and benefits, where the neoangiogenesis process, fibroblast activity, collagen storage and hydroxyproline concentrations are higher than without the use of amniotic membranes[7]. The effectiveness of using the amniotic membrane can be measured by the amount of collagen in the intestinal tissue that is anastomosed to. This amount of collagen can be measured both qualitatively and quantitatively. Measurement of the amount of collagen quantitatively carried out is by measuring the hydroxyproline levels in the tissue[10].

On this basis, we conducted a study on Wistar rats because their intestinal anatomy and histology structure is similar to that of humans. The use of fibrin glue against intestinal anastomoses as a wound barrier (sealant) which prevents bacteria from entering the anastomotic suture, while the role of dry amniotic membrane against anastomosis which besides having barrier properties (antibacterial) also has a growth factor component. It is against this background that this study was made, this study aims to compare the effect of amniotic membrane and fibrin glue on colonic anastomoses in the experimental conditions of intra-peritoneal infection in rats by looking at hydroxyproline levels in the anastomotic gut.

Method

This research is an experimental study on mice, because the experimental unit gets treatment. The design used is the Randomized Post Test Only Control Group Design so that the measurement of the variable is only done at the end of the study. Performed on Wistar rats when compared to humans, these mice have similar anatomical structures of the abdomen, liver and intestines.

Rats will be divided into three groups, each with the same sample size. The first group (I) anastomosis used a severed knot technique (control), the second group (II) performed anastomosis using a broken knot technique by adding dry amniotic membrane and the third group (III) performed anastomosis using a broken knot technique by adding fibrin glue. The target number of mice examined for each treatment was 9 mice. According to the minimum requirements of the Frederer formula. As an anticipation if there are mice that die, 2 mice are added for each treatment. So that a total of 33 rats were obtained, divided into 11 rats in group I, 11 rats in group II and 11 mice in group III.

Each mouse was assigned a number placed on the tail. When performing actions on experimental animals, operators are only given instructions by the scrambler to perform with or without amnion or fibrin glue. The scrambler was recorded without the operator's knowledge. When taking the results of the seventh day, the rats were sacrificed to measure the hydroxyprolin levels of the intestinal anastomotic tissue. The operator did not know whether the mice were previously given dry amniotic membrane or fibrin glue or not. During the examination process at the Clinical Pathology Laboratory, the examiner was only given the mouse number, but did not know whether the specimen was included in the group with or without amnion or fibrin glue. After the results were obtained, the randomizer was recorded. From the results of recording, data analysis was carried out.

Results

33 Wistar rats (Rattus norvegicus) were selected, aged 10-12 weeks, weighing 250 - 300 grams. All rats were subjected to fecal induced peritonitis (FIP) 1 day before colonic anastomosis surgery. Then divided into three groups, performed a left colon resection at a distance of 3 cm from the peritoneal reflection and performed anastomosis. Group I was anastomosed using a broken knot technique, group II was anastomosed by adding dry amniotic membrane and group III was anastomosed by adding fibrin glue. All mice were sacrificed on the 7th day, then the anastomotic segment was cut along 5 mm proximal and 5 mm distally, to check the hydroxyproline levels. The specimen was sent to the Laboratory of the Genetic Institute of Tropical Disease for tissue homogenate. Then sent to the clinical pathology section for examination of hydroxyproline levels. The data on the characteristics of the research sample are as illustrated in the table below. (Table 1). It was found that the mean age of rats in group I = 11.00 weeks (± SD = 0.866), the mean age of rats in group II = 10.67 weeks (± SD = 0.707) and the average age of rats in group III = 10.78 weeks (± SD = 0.833). Then from the data, the data normality test was carried out. In the normality test of the Shaphiro-Wilk test data (n <50), the research sample data for the age of the rats had p> 0.05 (group I = 0.073, group II = 0.085 and group III = 0.064).

Table 1. Data on the Characteristics of Research Subjects

Parameter	Average Interrupted Suture (I)	Average Interrupted Suture + Dry Amniotic Membrane (II)	Average Interrupted Suture + Fibrin Glue (III)	P Value
Age	11,00 weeks	10,67 weeks	10,78 weeks	0,675
Weight	276,67 grams	277,78 grams	277,78 grams	0,978

This indicates that the rat age data in the three sample groups has a normal distribution. ANOVA test on sample body weight shows that p> 0.05 (0.675) means that there is no statistically significant age difference in the three treatment sample groups. The average body weight of rats in group I = 276.67 grams (± SD = 14.142), the average body weight of rats in group II = 277.78 grams (± SD = 12.019) and the average body weight of rats in group III = 277 , 78 grams (± SD = 12.019). In the normality test of the Shapiro-Wilk test data (n <50), the sample data for the study's body weight of rats had p> 0.05 (group I = 0.618, group II = 0.586 and group III = 0.076). This indicates that the rat body weight data in the three sample groups have a normal distribution. ANOVA test on sample body weight showed that p> 0.05 (0.978) means that there is no statistically significant difference in body weight in the three treatment sample groups.

Table 2. Hydroxyproline Measurement Results

Groups	Average	Standard Deviation
Simple Interrupted (I)	2157,41	478,60
Simple Interrupted + Dry Amniotic Membrane (II)	2887,40	688,49
Simple Interrupted + Fibrin Glue (III)	2224,59	416,63

The average difference in hydroxyproline levels per 1 milligram of tissue was that in group I = 2157.41 (± SD = 478.60), the average hydroxyproline levels for group II = 2887.40 (± SD = 688.49) and The average hydroxyproline level for group III = 2224.59 (± SD = 416.63). In the normality test of the Shapiro-Wilk test data (n <50), the research sample data for the hydroxyproline content had p> 0.05 (group I = 0.884, group II = 0.076 and group III = 0.224). This indicates that the data on hydroxyproline levels in the three sample groups have a normal distribution. Then the statistical test used is the parametric test. ANOVA test on the hydroxyproline level of the sample showed that p <0.05 (0.015) means that there is a statistically significant difference in hydroxyproline levels in the three groups of the treatment sample.

Table 3. Post Hoc ANOVA Test

	Group	Intevention	Mean Difference	P Value
Hydroxyproline Levels	Simple Interrupted (I)	Simple Interrupted + Fibrin Glue	-67,17	0,962
		Simple Interrupted + Dry Amniotic Membrane	-729,98	0,022
	Simple Interrupted + Dry Amniotic Membrane (II)	Simple Interrupted	729,98	0,022
		Simple Interrupted + Fibrin Glue	662,81	0,040
	Simple Interrupted + Fibrin Glue (III)	Simple Interrupted	67,17	0,962
		Simple Interrupted + Dry Amniotic Membrane	-662,81	0,040

Post Hoc ANOVA test showed that hydroxyproline levels in treatment group II (Sew Interrupted + MAK) had the highest Mean Difference (positive compared to other groups), with a significance test of p <0.05 à meaning hydroxyproline levels in treatment group II (Interrupted sutures + MAK) were highest and this was statistically significant. Meanwhile, the treatment group I (Sew Interrupted) and treatment group III (Sew Interrupted + Fibrin Glue) did not have a large mean difference and were not statistically different (p> 0.05). In conclusion, treatment group II (Sew Interrupted + MAK) had the highest levels of hydroxyproline and this

was statistically significant.

Discussion

A total of 33 Wistar rats were divided into three treatment groups, 11 in group I were anastomosed using a broken knot technique, 11 in group II were anastomosed by adding dry amniotic membrane and 11 in group III were anastomosed by adding fibrin glue. One day before the operation, 33 rats were subjected to faecal induced peritonitis (FIP), after 24 hours all the rats were performed laparotomy and found cloudy peritoneal fluid accompanied by reddish intestines then performed left colon resection and anastomosis according to the treatment group. In group I, 1 mouse died on the first day and 1 mouse died on the third day. In group II, no mice died. In group III, 1 rat died on the second day. Due to the limited amount of fresh lysis buffer reagent, the number of mice examined is only a minimum requirement. From rats living on day seven, 9 rats from group I, 9 mice from group II and 9 mice from group III were randomly taken. On day 7, 27 rats were subjected to relaparotomy, obtained a cloudy peritoneal fluid and a little slough that adhered to the intestinal wall, in group I the intestinal part that was subjected to anastomotic resection was still incompletely dense intestinal tissue while in groups II and III intestinal tissue the anastomosis is done is closed completely.

This study shows that the use of dry amniotic membranes can increase hydroxyproline levels in anastomotic tissue which correlates with an increase in the amount of collagen quantitatively because the results were statistically significant on hydroxyproline levels. This proves that the role of dry amniotic membranes in intestinal anastomosis apart from having barrier (antibacterial) properties[7] also has a growth factor component[8] While the role of fibrin glue in intestinal anastomosis is only as a wound barrier (sealant) which prevents bacteria from entering the suture. anastomosis[4]. The amniotic membrane has other features, namely that it contains large amounts of growth factors, cytokines, and extracellular matrix, so it plays an important role in wound healing[9]. Small amounts of collagen will result in poor anastomotic healing thereby increasing the risk of anastomotic leakage[11]. The healing process for intestinal anastomosis is different from the healing process in other tissues. Collagen in the digestive tract is produced by fibroblasts and smooth muscle cells. Collagen synthesis peaks at day 5 to day 7 after intestinal anastomosis [7].

In this study, administration of dry amniotic membrane was significant in intestinal anastomosis in peritonitis conditions where various mechanisms of amniotic membrane action were proposed to explain the anti-inflammatory action of the amniotic membrane, such as inducing apoptosis, inhibiting chemotactic activity of polymorphonuclear neutrophils (PMN) and macrophages. The anti-bacterial properties of the amniotic membrane are possible due to elements such as interferon, lysozyme, transferrin, progesterone, immunoglobulin, and globulin B1c / B1a that are present in the amniotic membrane[7]. The results of this study are in accordance with research conducted by Uludag, et al., The use of amniotic membranes in colon anastomosis provides significant effects and benefits, where the neoangiogenesis process, fibroblast activity, collagen deposits and hydroxyprolin concentrations are higher than without the use of amniotic membranes[7].

Conclusion

Based on the results of the study, it can be concluded that the use of the amniotic membrane provides a better colonic anastomosis compared to the use of fibrin glue in conditions of intra-peritoneal infection, where there is an increase in hydroxyproline levels in the intestinal anastomotic tissue wrapped in dry amniotic membrane with statistically significant results.

Ethical Clearance: Taken from Ethical Committee of Animal Research, Faculty of Veterinarian, Airlangga University

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Conflict of Interest : Nil

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