

RESEARCH ARTICLE

Differences in the Effect of Using Sterile Water for Irrigation and Normal Saline Washing to Peritoneal Adhesion Post-Laparotomy on White Rats

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Abstract

BACKGROUND: Peritoneal adhesion is a side effect of abdominal surgery that often occurs. Many efforts were made in abdominal surgery to prevent or minimize the occurrence of this adhesion. One way to reduce the risk of bowel adhesions is through washing peritoneum method, so the differences in the use of washing solution on the incidence of adhesions after laparotomy surgery need to be investigated. The objective of this study is to determine differences in the effect of using sterile water and normal saline for washing irrigation to peritoneal adhesions after laparotomy in white rats.

METHODS: This was an experimental study. Thirty-two sample of white rats were divided into two groups, group A and Group B, each group consists of 16 rats. Group A got washing using normal saline and group B got washing using sterile water for irrigation in laparotomy procedure to assess the peritoneal adhesion microscopically after relaparotomy.

RESULTS: This study was conducted in April-May 2016. The founding of peritoneal adhesion after laparotomy in white rats using sterile water for irrigation in grade 1 were seven samples, grade 2 were five samples and grade 3 were four samples. Meanwhile, the founding of peritoneum adhesion after laparotomy using normal saline grade 1 are two samples, grade 2 are five samples, and grade 3 are nine samples. Statistically, significant differences were found ($p < 0.05$).

CONCLUSION: Some risk factors can lead to adhesion, such as trauma surgery, tissue ischemia, infection blood and foreign body irritating. Intraperitoneal irrigation with sterile water for irrigation is better than the use of normal saline in preventing peritoneal adhesion.

KEYWORDS: peritoneal adhesions, sterile water for irrigation, normal saline

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Introduction

Peritoneal adhesion is one of the side effects that often occur after abdominal surgery.(1) Most of these conditions don't cause problems, but about of 5% of peritoneal adhesion can cause problems such as small bowel obstruction (SBO), infertility, pelvic and abdominal pain. These problems can

be a major factor in rising health care costs. Therefore, because of considerable morbidity and expensive treatment are required following a peritoneal adhesion, many attempts were being made to prevent or minimize the occurrence of these adhesions.(2)

Peritoneal adhesion is a complex process, and the etiology of the event is not certain. However, some risk factors can lead to adhesion, such as trauma surgery, tissue

ischemia, infection blood and foreign body irritating.(3) Some procedures that exist today in preventing or minimizing adhesion are: 1) the prevention of deposition of fibrin, 2) eliminate the exudates fibrin from the peritoneal cavity, 3) prevention of the proliferation of fibroblasts, and 4) the placement of barrier to prevent contact of the peritoneum to peritoneum during the formation of mesothelium.(4)

At the stage of laparotomy surgery procedures, before the closing of peritoneal, the abdominal washing needs to be done, generally by using a normal saline approximately 500-1,000 ml or as needed.(5) It aims to rid the remnants of blood and other fluids and the remaining pieces of tissue to avoid complications of adhesions after surgery.(6) Previous research proves that normal saline results in a decrease in fibrinolytic capacity and unfavorable influence on the structure and metabolism of peritoneal mesothelial cells. In addition, in another study also found that normal saline causes dysfunction of the mesothelial cells that can accelerate the formation of peritoneal adhesions.(7)

Sterile water for irrigation is sterile water with the chemical formula H_2O without other additives. The fluids are hypotonic, nonpyrogenic, and non-ionic. It is widely used for irrigation in the procedure endourological (8), so that the resected area remain bright and not covered in blood as well as during their occupational obtained no side effects.

The degree of adhesion is used to assess the severity of adhesions macroscopically and microscopically. The macroscopic degree of adhesion that occurs in normal saline washing is more likely causing peritoneal adhesions than sterile water for irrigation. Based on this fact, we did research to study differences in the effect of using sterile water for irrigation and normal saline washing to peritoneal adhesion post laparotomy on white rats from the degree of adhesion with the microscopically.

Methods

This was an experimental laboratory study, using post-test only control group design. The sample size was calculated using the formula Frederer, as follow:

$$\begin{aligned}(n-1)(k-1) &\geq 15 \\ (n-1)(2-1) &\geq 15 \\ \mathbf{n} &= \mathbf{16}\end{aligned}$$

n : number of sample required
k : the number of treatment groups

All the experimental animals were observed for one week for adaptation to their meal and environment as well as to ensure their health. Later, in the second week, they were divided into two groups. Anesthesia process was conducted by using ketamine anesthesia intramuscular, the dose of 5-10 mg/kg of body weight. Actions performed aseptically using a sterile instrument. Disinfection used 10% povidone iodine and 70% alcohol.

Then laparotomy with a 4 cm incision media was deepened to penetrate the peritoneum. Later, peritoneal was opened and washed intra-abdominally using 5 mL of normal saline in group A and then covered in layers by stitching the peritoneum with absorbable multifilament 3.0. For group B, the same procedure was done, but intra-abdominal washing was done using sterile water for irrigation® and by stitching the skin with nonabsorbable multifilament 2.0.

Results

The research on the effect of differences in water for washing using sterile water for irrigation and normal saline was conducted in April-May 2016, with 32 samples of Wistar strain white rats were divided into two groups. The founding of peritoneal adhesion after laparotomy in white rats using sterile water for irrigation in grade 1 were seven samples, grade 2 were five samples and grade 3 were four samples. Meanwhile, the founding of peritoneum adhesion after laparotomy by abstersion using normal saline grade 1 are two samples, grade 2 are five samples and grade 3 are nine samples.

Mann-Withney test results obtained p -value=0,047 ($p<0,05$), which means that there are differences in the effect of washing using sterile water for irrigation and normal saline to peritoneal adhesions after laparotomy in rats. Figure 1, Figure 2 and Figure 3 show the picture of peritoneal adhesion microscopically.

Discussion

Peritoneal adhesions is an abnormal fibrous adhesion (connective tissue) that occurs between adjacent surfaces of the parietal and visceral peritoneum. The presence of adhesion can cause adhesions between organs intraperitoneal, for example between intestinal loops close, and with the

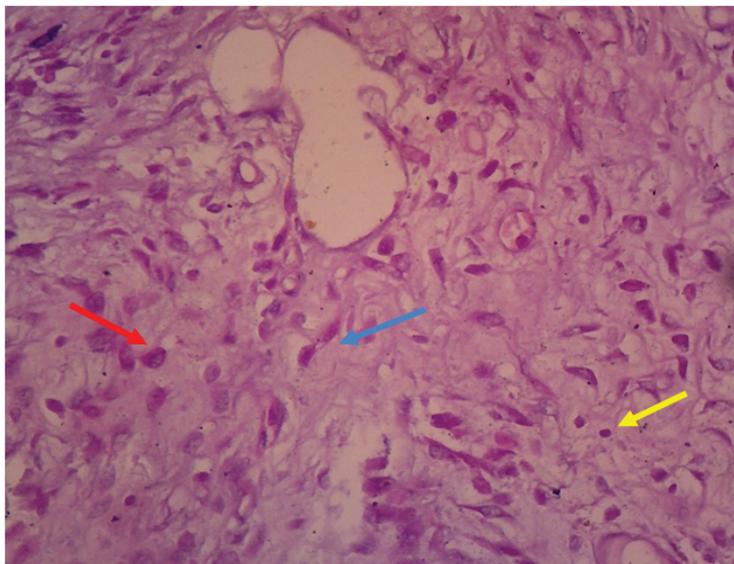


Figure 1. Histopathological picture of peritoneal tissue adhesion with 400x magnification. HE (Grade 1): the red arrow shows the plasma cells, the blue arrow shows histiocytes and the yellow arrow shows lymphocytes.

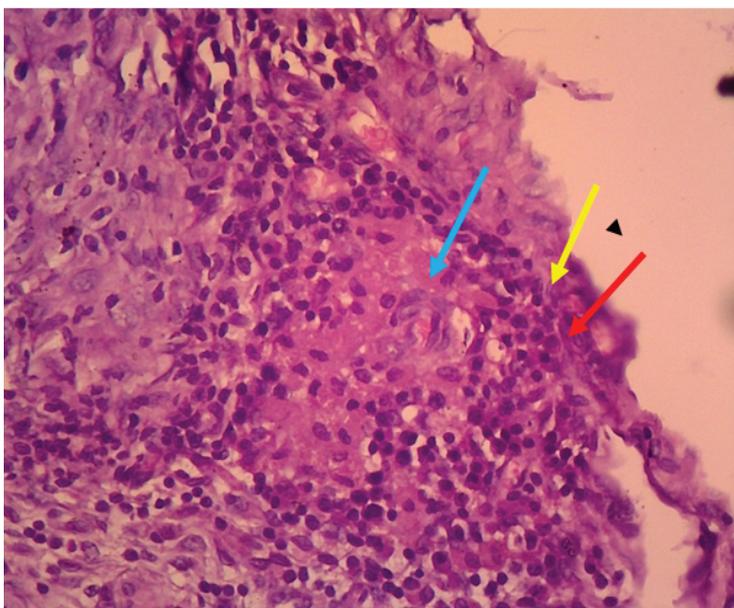


Figure 2. Histopathological picture of peritoneal tissue adhesion with 400x magnification. HE (Grade 2): the red arrow shows the plasma cells, the blue arrow shows histiocytes and the yellow arrow shows lymphocytes.

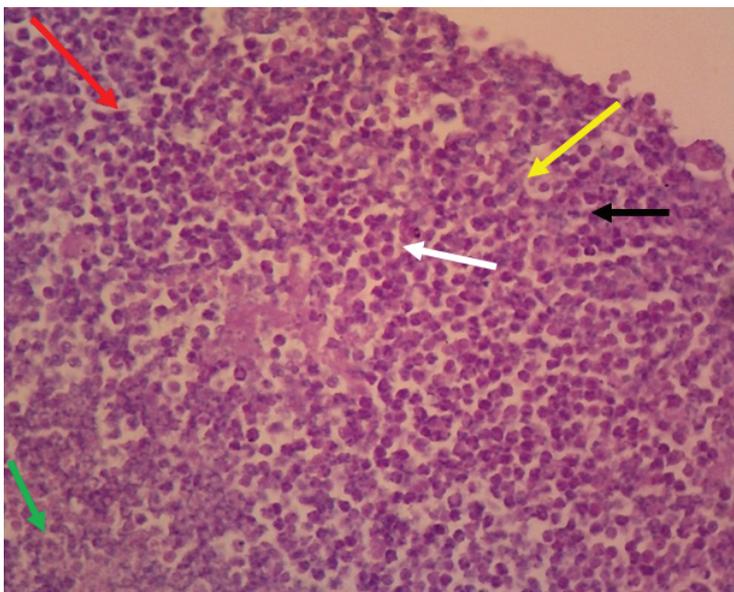


Figure 3. Histopathological picture of peritoneal tissue adhesion with 400x magnification. HE (Grade 3): the red arrow shows plasma cells, the green arrow shows the necrotic tissue, the yellows arrow shows lymphocytes, the white arrow shows eosinophils and the black arrow shows core fragment neutrophils.

wall parietal peritoneum. Peritoneal mesothelium is a layer of cells with basement membrane complex supported by connective tissue rich in blood vessels. Consisting of parietal peritoneum lining the abdominal cavity, diaphragm and retroperitoneum and peritoneum visceral organs that cover the entire surface of the organs in the abdomen. The peritoneum is divided into two parts, namely connective tissue histologically and mesothel. Connective tissue or sub mesothel network consists of an extra cellular matrix consisting of glycoproteins, glycosaminoglycan, proteoglycans, collagen, fibroblasts, macrophages and mast cells, also fat tissue. In subserous, there are blood vessels, nerves, and lymphatics. Mesothel layer consists of loose mesenchymal tissue bounded by the basement membrane.(9)

The healing process is very different between peritoneum and skin. The re-epithelialized skin will go through the stages of proliferation of epithelial cells from the periphery toward the center of the wound, while the peritoneum mesothelialization will take place simultaneously, and does not depend on the size of the wound; the mesothel cell is growing new islands which will proliferate and form layer of cells. So that small and large wounds on peritoneum will experience epithelization at the same time at the same speed, about 4-7 days to the parietal peritoneum and visceral peritoneum. Normally, wound healing occurs in the absence of adhesion formation.

Trauma to the peritoneal mesothelium tissues causing inflammatory reactions as the body's response. At the cellular level, prostaglandins are released and activating inflammatory components such as neutrophils, macrophages, mast cells, basophils, platelets, endothelial cells and leukocytes lymphocytes. Mast cells release inflammatory mediators such as histamine, serotonin, lysosomal enzymes, chemotactic factors, and cytokines and reactive oxygen metabolites to kill bacteria, eliminate foreign bodies and improve body functions both in anatomy and physiology.(10) Histamine causes an increase of vascular permeability peritoneum produces rich transudation fibrinogen into the peritoneal cavity and causes neutrophils to enter the wound area. The main function of neutrophils are phagocytic cells, destroy bacteria and help clear the dead tissue. Macrophages secrete Transforming Growth Factor Beta (TGF- β) that stimulates fibroblast proliferation and regulation of the mesothelium cells to produce fibrin. On the second day of macrophages will form a layer on the traumatized peritoneum. Fibrin deposits will be formed between 48 to 72 hours after laparotomy. In the

third and fourth days, there are infiltration and proliferation of fibroblast. At this time also been a proliferation of endothelial cells in the neovascularization process, the process of re-epithelialization of peritoneal tissue.(1) Fibrinolysis is started at least three days after the trauma and increased rapidly on the eighth day of the mesothelium cell regeneration in the complex. When fibrinolysis is running normally, in fourth and fifth days mesothelium cells will grow along the wound and cover the damage in total. Starting on the fifth and sixth day and it will decrease, and on the eighth day the mesothelium cells shall cover the wound and regenerate complex occurs. When the fibrinolytic system fails in lysis process, the fibrin connective tissue will then formed connective tissue that persistent.(3)

The degree of adhesion is used to assess the severity of adhesion. It is useful for research and clinical. This degree is assessed of both macroscopically and microscopically. While the degree of adhesion microscopically can be divided into scores degree as follow absence, mild inflammation, moderate inflammation and severe inflammation (Table 1).(11)

Based on the results shown in Table 2, there was a difference effect of the use of sterile water for irrigation and normal saline as a washing solution. The majority of the result showed the degree of adhesion in grade 1 (7 rats) when using sterile water for irrigation as abdomen's washing solution. Meanwhile, most of the result showed the degree of adhesion in grade 3 (9 rats) when normal saline was used.

The results of this study are supported by previous studies, which show that normal saline is affecting the decrease in fibrinolytic capacity and unfavorable influence on the structure and metabolism of peritoneal mesothelial cells.(7) In addition, another study also found that normal saline causes dysfunction of the mesothelial cells that can accelerate the formation of peritoneal adhesions.(5) Dysfunction of the mesothelial cells causing an inflammatory reaction as the body's response to the healing process.

Sodium chloride is a crystalloid fluid that can be used as a mechanical barrier to prevent fluid adhesion. Absorption of water and electrolytes from the peritoneal cavity is very fast, average 30-50 mL/hour. Isoosmolar NaCl is absorbed in less than 24 hours. Normal saline is a fluid isotonic, liquid that has the same concentration of solute with a concentration within the cell. Because there is no difference in gradient between extracellular and intracellular tissue, then there is no movement of molecules across the cell membrane. The

Table 1. The Degree of Adhesion.

Score	Degree	Description
0	Absence	There were no cells inflammatory cells (0%) at the location of attachment.
1	Mild inflammation	Obtained inflammatory cells (1-15%) the entire field of view at the location of attachment.
2	Moderate inflammation	Obtained inflammatory cells (16-49%) the entire field of view at the location of attachment.
3	Severe inflammation	Obtained inflammatory cells (50-100%) the entire field of view at the location of attachment.

Table 2. Effect of washing using sterile water for irrigation and normal saline to peritoneal adhesions in white rats

Degree of Adhesion	Treatment		Total	p-value (Man Whitney)	Sign
	Sterile Water	Normal Saline			
Grade 1	7	2	9		
Grade 2	5	5	10	0.047	S
Grade 3	4	9	13		
Total	16	16	32		

movement of molecular diffusion occurs in two directions at the same speed so that the cells do not lose or excess fluid.(12) Therefore, neovascularization process happens, so it results in an increase in fibrous tissue. Crystalloid fluid does not decrease the incidence of postoperative adhesions in research conducted. Some other studies have been done to prove that normal saline decrease cell viability and fibrinolysis activity mesothel peritoneum, so it concluded that normal saline is a predisposing factor for the formation of peritoneal adhesions.(7)

Sterile water for irrigation® is sterile water with the formula H₂O without other additives. The liquid with a PH of 7.0 is used for irrigation, which is hypotonic, nonpyrogenic and non-ionic. This fluid is used for irrigation during surgery, clean the blood at the site of surgery, moisten gauze, wound irrigation, soak and wash medical equipment. It is widely used for irrigation on an endourological procedure.(8) Hypotonic fluid is a liquid with a lower concentration of solute than others, so the water moves easily into the cells. By placing cells in a hypotonic solution, osmotic pressure causes the tissue drain the water into the cells, causing cells swelling.(12) Since the cells are swelling, neovascularization

did not happen, so it does not result in an increase in fibrous tissue. Fibrinolysis occurred, and peritoneal adhesion are decreased.(13)

Conclusion

Some risk factors can lead to adhesion, such as trauma surgery, tissue ischemia, infection blood, and foreign body irritating. Intraperitoneal irrigation with sterile water for irrigation is better than the use of normal saline in preventing peritoneal adhesion.

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