RESEARCH ARTICLE OPEN ACCESS

Polymeric Chitosan Gel Modulates Inflammatory Responses to Prevent Peritoneal Adhesion in Rats

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Received 10 January 2023
Accepted 28 January 2023
Available online on 30 January 2023

ABSTRACT

Background: Peritoneal adhesions are a pathological response to injury that connects adjacent structures that are caused by inflammatory response such as IL-1 and IL-6. The current strategies to minimize or prevent peritoneal adhesions are very limited. Chitosan was found to have an anti-inflammatory effect. However, chitosan hydrophobic properties give poor solubility in physiological solvents such as peritoneal fluid. **Objective:** Our study aims to investigate the effect of polymeric chitosan gel in the prevention of peritoneal adhesion through modulating IL-1 and IL-6 after laparotomy. **Methods:** Thirty rats were divided into a control positive, control negative and treated group evenly. The laparotomy model was done with proper procedure. Ileum were scratched using cytobrush to mimic peritoneal adhesions. The defect was covered with olive oil and Mediclore® in control positive and treated group respectively. The control negative group was not receiving any treatment. After 14 days, IL-1 and IL-6 were analyzed using ELISA from peritoneal fluid serum and macroscopic observation of peritoneal adhesion were made. **Results:** All animals in the treated group showed a filmy peritoneal adhesion compared to control negative and control positive group. There is a significantly lower IL-1 and IL-6 levels compared to other groups. **Conclusion:** In conclusion, polymeric chitosan gel prevents peritoneal adhesions through modulation of inflammatory cytokines after laparotomy in rats.

Keywords: Chitosan, IL-1, IL-6, Peritoneal Adhesions, Laparotomy

INTRODUCTION

Peritoneal adhesions are a pathological response to injury that connects adjacent structures.[1] It can range from a thin film of connective tissue to a thick fiber band including blood vessels and nerves to a tight connection between the near surfaces.[2] Peritoneal adhesions may complicate bowel obstruction, abdominal pain, or even infertility as it connects reproductive organs.[3] Peritoneal adhesions happen because of inflammation after open surgery.[4] Laparotomy may lead to high inflammatory reaction around the peritoneal and internal organs; hence it may inhibit the fibrinolytic system of the abdominal organ and continuously produce and store fibrin around the peritoneal may resulting in peritoneal adhesions.[5,6]

Cellular mediators contained in the peritoneal fluid can influence inflammation over organs inside the peritoneum cavity.[7–9] Interleukin-1 (IL-1) were an important cytokine that secrete in the early

phase of wound healing after surgery since macrophages were activated in the peritoneal.[10–12] While interleukin-6 (IL-6) was expressed by macrophage, and it was influenced by IL-1 during the inflammation phase.[13–15] Both cytokines were responsible for fibrin and extracellular matrix formation inside the abdomen.[16–18]

The current strategies to minimize or prevent peritoneal adhesions are very limited. A short and clean surgery may result in a lower inflammatory response.[19,20] Although, some harder cases need a longer time and a larger area of operation which may increase the risk of peritoneal adhesions.[21–23] The intraoperative biological agent applied directly to the surgical site may reduce peritoneal adhesion by lowering the inflammation and preventing direct attachment of the peritoneal adhesions.[24–27] However biological agents such as Interceed, Adept, or Suprafilm may contain important side effects such as allergic reactions and damage to the anastomosis.[28]

Chitosan is a deacetylating chitin that contains high adsorption ability.[29] Chitosan is widely used as a drug carrier since its biodegradability properties.[30] Chitosan also provides anti-inflammatory activity and promotes the proliferation phase, thus may be an effective agent for preventing peritoneal adhesions.[31–33] However, chitosan hydrophobic properties give poor solubility in a physiological solvent such as peritoneal fluid.[34]

A carbohydrate polymer of chitosan has been widely used as a drug delivery system. It serves biocompatible and hemocompatible water-soluble drugs.[35–37] It offers possibilities to be used as a drug delivery system in oral or intranasal.[38] However, polymeric chitosan itself was not used as a prevention biochemical agent to prevent peritoneal adhesion after laparotomy. Our study aims to investigate the effect of polymeric chitosan gel in the prevention of peritoneal adhesion through modulating IL-1 and IL-6 after laparotomy.

MATERIAL AND METHODS

Material

A polymeric chitosan gel named Mediclore® was purchased from Daewong, Indonesia. A 5 ml syringe of Mediclore® was used as anti-adhesion agent in this study. Standard manufacturer olive oil was used for the positive control group.

Animal Models

Thirty Sprague–Dawley rats were divided into three groups (n = 10 per group) a control negative, control positive and treated group respectively. Rats with similar body weights (220 ± 10 grams) had been caged in a standard laboratory animal with controlled temperature and humidity. The rats were placed in a 12-hours light-dark cycle and fed with standard food and water. After three days of adaptation, the laparotomy model was done with proper procedure. Rats were shaved in the anterior of the abdomen and subsequently were anesthetized with 1% sodium pentobarbital (0.4 mL/100 g) intraperitoneal and placed in a supine position. To open the abdomen, a 4 centimeters incision was made in the midline. A 2 centimeters defect in the ileum was made using a cytology brush to provoke intraperitoneal inflammation (Figure 1). Rats in the control negative group were not treated with any biological agent and the abdomen was closed directly to prevent longer operation time. While rats in the control positive group were treated with 0.05 mL/cm of olive oil to mimic the hydrophobic properties in the treated group. In the treated group, the defect was covered with Mediclore® with a dose of 0.05 mL/cm of a defect. The abdominal wall and the skin were closed directly using a 4–0 polyglycolide suture (T-VIO, Triton, Indonesia). After 14 days, the peritoneal fluids were taken, and a laparotomy was performed to investigate peritoneal adhesions. This study has been approved by the Ethical Committee of Sultan Agung Islamic University.

Peritoneal adhesion measurement

Peritoneal adhesions were measured macroscopically according to the scoring systems by Lauder et al.39 The Lauder scoring system (Table 1) considers the number, strength, and distribution of adhesions in a single score. Two independent inspectors were done the scoring of peritoneal adhesion to prevent measurement bias.

Table 1. Peritoneal adhesion scoring system according to Lauder et al.[39]

Score	Description
0	No adhesions
1	Thini filmy adhesions
2	More than one thin adhesion
3	Thick adhesion with focal point
4	Thic adhesion with planar attachment
5	Very thick vascularized adhesions or more than one planar attachment

Inflammatory cytokines measurement

The effect of polymeric chitosan gel on inflammatory cytokines including IL-1 and IL-6 in peritoneal fluid serum was determined using an ELISA kit (Termofisher, USA) following the manufacturer's standard protocol.

Statistical analysis

The data are presented as means ± standard error of the mean. The One-Way ANOVA was used to analyze inflammatory cytokines of IL-1 and IL-6 between the control and treated groups. LSD Post hoc test was used to examine the difference between groups. Peritoneal adhesions scoring was tabulated into a cross tab and Pearson Chi-Square was used in this study. $P < 0.05$ was considered significant. All the statistical analyses were undertaken using SPSS 22.0 software (Chicago, USA).

RESULTS

Peritoneal Adhesion

In the control negative group, all animals showed peritoneal adhesions, which were rated with the maximum Lauder score by our investigators. While most of the control positives treated with olive oil showed a thick adhesion. In contrast, all animals in the treated group showed a filmy peritoneal adhesion or no adhesion at all. A cross-tabulation (Table 2) between the control negative, positive, and treated groups showed there is a significant difference between groups using the Pearson Chi-Square test (P= 0.000).

Table 2. Cross tabulation showing macroscopic assessment of peritoneal adhesions.

Group	**Score**					**Pearson Chi-Square**
	1	**2**	**3**	**4**	**5**	
Control	0	0	0	0	10	0.000
Olive Oil	0	2	6	2	0	
Chitosan	7	3	0	0	0	

IL-1 and IL-6 assays

Evaluation from peritoneal fluid serum using ELISA showed that there is a significant difference IL-1 levels between all groups (P<0.001). Group treated with Mediclore® showed the lowest IL-1 levels compared to control group (P<0.001) and group treated with olive oil (P = 0.015) (Figure 1). In accordance with the results, IL-6 levels also showed a significant difference between treated group

and other groups (P<0.001). Group treated with Mediclore® showed the best control at inflammation by scoring the lowest IL-6 levels compared to control negative (P<0.001) and positive group (P=0.012). Meanwhile no significant difference was found between the control negative group and group treated with olive oil (P=0.052) (Figure 2).

Figure 1. A defect was made in terminal ileum using cytology brush to provoke peritoneal adhesions.

DISCUSSION

Postoperative intraabdominal adhesions are an important cause of long-term morbidity and therefore there have been many studies on the prevention of adhesions in the scientific literature.[40] Postoperative peritoneal adhesions occur because of damage to any area in the peritoneum.[41] A prolonged inflammation by secretion of IL-1 and IL-6 may result in fibrin formation. Fibrin forms bands between other peritoneal surfaces that encounter this area. Fibrin bands and the hyaluronic acid-rich matrix that fills the gap between them provide a very suitable environment for collagen synthesis and form an adhesion.[42] Abdominal adhesions can cause chronic pain, intestinal obstruction, fistulas, and infertility.[28]

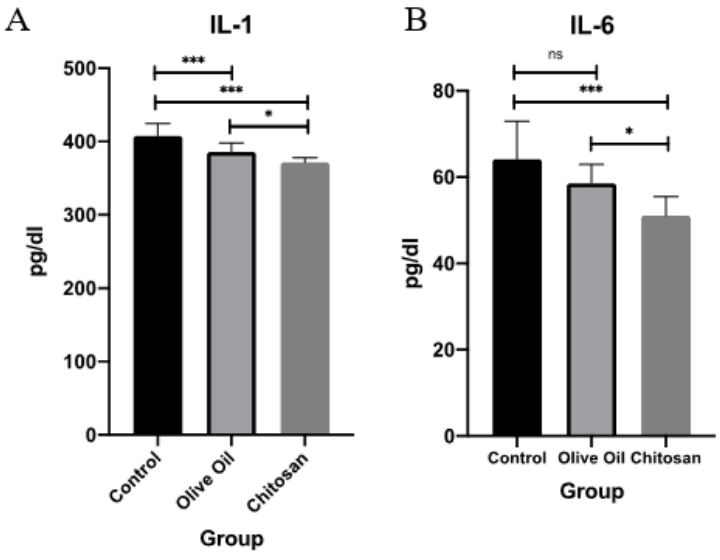

Figure 2. A bar chart showed that IL-1 and IL-6 in the treated group was significantly lower compared to the control group.

A method to prevent intraabdominal adhesion formation will eliminate the morbidity caused by adhesion.[43] Several techniques, materials, and agents have been tried to prevent adhesions such as surgical methods, minimally invasive and laparoscopic techniques, and pharmacological agents targeting the inflammatory response and fibrin formation and barrier between organ surfaces.[20] Although useful techniques or agents were found, no complete success was achieved, and the results were not reflected in surgical practice except for adhesion barriers.[19]

Adhesion barriers are usually applied directly to the surgical site using biological materials or gel substances.[40] This agent may reduce peritoneal adhesion by preventing direct attachment in the peritoneum.[42] However, other biological materials used to prevent inflammation inside the peritoneum may result in allergic reactions and reduce wound healing by inhibiting anastomosis in the wound site.[25,44] Chitosan is a biological material from deacetylating chitin that is widely used in drug carriers because it contains high adsorption ability.[36,37] However, Chitosan has also been used as an anti-inflammatory agent. With its properties, chitosan may be used as one of the biological agents to prevent peritoneal adhesion.[31,45]

As shown in our study, rats in the control negative group, which receive no treatment after laparotomy showed a higher degree of peritoneal adhesion and inflammatory cytokines in IL-1 and IL-6 levels. This result showed that our animal model was a success to promote peritoneal adhesion by provoking inflammation inside the peritoneum. According to a previous studies, a similar result was found using the identical animal models method.[46–48]

Animal experiments in the control positive group showed that the application of olive oil after laparotomy gives a better adhesion level compared to the control negative group. However, there is no significant difference in IL-6 levels compared to the control negative group. This effect demonstrated that olive oil may act as a physical barrier to prevent direct attachment between two wounded sides inside the peritoneum but not as an anti-inflammatory agent.[49,50] A significant difference was found in IL-1 levels between control negative and control positive. We hypothesized this result may come from numerous phenolic compounds in olive oil that exert anti-inflammatory effects.[51,52]

This study also showed that the lowest adhesion level was found in the group treated with chitosan gel. Furthermore, it is supported by the lowest IL-1 and IL-6 levels compared to control positive and negative groups. We suggest that chitosan gel act not only as a barrier agent but also to inhibit inflammatory cells at the wound site, therefore, preventing collagen synthesis and adhesion formation inside the peritoneum. Chitosan reduces collagen deposition through modulation of TNF-a, IL-1, and IL-6 inflammatory cytokines which represents our result.[53–55] These findings were hypothetically explained through the activation of the NLRP3 pathway.[56,57] NLRP3 activation directly leads to inflammasome activation and release of the inflammatory factors IL-1β and PGE2.[58,59] Our study used inflammatory cytokines assay from the peritoneal fluid sample which may represent local inflammation markers after laparotomy, thus will give an accurate representation of the result.[58,60] Another reports found that chitosan amino groups may stimulate fibroblast and promote wound healing.[34,55,56]

The major pathway to inhibit peritoneal adhesions is accelerating wound healing and decreasing inflammation in the peritoneum.[61] While hemostasis and infection may be involved to slow down the wound-healing process.[62] Therefore, some reports showed that chitosan has anti-bacterial activity by causing damage to the outer membrane of the cell thus inhibiting bacterial growth.[33,36] In addition, Chitosan functions to stop bleeding by creating a three-dimensional network to capture and aggregate red blood cells to create coagulation.[63-65]

The limitation of this study, we only check peritoneal adhesion by macroscopic observation, which may give various interpretations according to the investigators. Although, our study had two

independent investigators to prevent such things. A prominent parameter such as fibrin deposition, collagen, or histological assay may give a better understanding of this study.

CONCLUSION

In conclusion, polymeric chitosan gel prevents peritoneal adhesions through the modulation of inflammatory cytokines after laparotomy in rats.

FUNDING

None

ACKNOWLEDGEMENTS

This project was supported by a grant from the Faculty of Medicine Sultan Agung Islamic University, Semarang, Indonesia. The authors declared that there is no competing interest in publishing this study.

AUTHORS' CONTRIBUTIONS

FRM wrote the first manuscript and did the animal models. VME supervised the animal models and manuscript writing. DAA, RS, and IYK prepared the animal models and collected the data.

COMPETING INTERESTS

The authors declare that there is no conflict of interest.

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