



RESEARCH ARTICLE

Peritoneal lavage with sterile water reduces IL-1 levels and postoperative adhesions following laparotomy in rats

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ABSTRACT

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Peritoneal lavage with normal saline is common in abdominal surgeries, yet recent studies indicate that it increases intraperitoneal adhesion risks. This study compares the effects of peritoneal lavage with normal saline versus sterile water on interleukin-1 (IL-1) levels and intraperitoneal adhesion following laparotomy in rats. In this post-test control group study, 20 adult Wistar rats were subjected to laparotomy before being randomly divided into two groups to receive either intraperitoneal lavage with normal saline (0.9% NaCl) or sterile water. After 7 days, IL-1 level and degree of adhesion were evaluated. The saline group had higher adhesion levels (4 rats with grade 4, 5 with grade 3, 1 with grade 2) than the sterile water group, which had lower levels (2 rats with grade 3, 5 with grade 2, 3 with grade 1). There was a significant difference in IL-1 levels between the sterile water group ($37,111.6 \pm 6,535.61$ pg/ml) and the normal saline group ($57,456.3 \pm 10,583.41$ pg/ml). There was a significant correlation between IL-1 levels and adhesion grade ($p=0.008$). Intraperitoneal lavage with sterile water results in significantly lower adhesion grade and IL-1 levels than normal saline, suggesting its potential for reducing postoperative adhesions. Further studies are needed to elucidate the mechanism of reduced adhesion and inflammation associated with sterile water lavage.

1. Introduction

Peritoneal adhesion represents an abnormal fibrous connection between adjacent surfaces of the parietal and visceral peritoneum, which can manifest as thin layers of connective tissue, thick fibrous bridges with blood vessels and nerve tissues, or direct contact between two organ surfaces (Octaviani *et al.*, 2017). Such adhesions predominantly occur following surgical procedures in the peritoneal cavity, with a prevalence

ranging from 63% to 97% (Tabibian *et al.*, 2017). Postsurgical peritoneal injuries release proinflammatory mediators, activate the coagulation cascade, and result in fibrin formation. The balance between tissue-type plasminogen activator (tPA) and plasminogen activating inhibitor-1 (PAI-1) influences fibrin deposition and degradation. When tPA levels exceed those of PAI-1, fibrin is completely degraded through fibrinolysis, preventing adhesion formation (Moris *et al.*, 2017).

Conversely, lower tPA levels relative to PAI-1

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impair fibrinolysis, leading to adhesion formation. Surgical procedures release proinflammatory mediators like interleukin-1 (IL-1), which escalate the incidence of adhesions by increasing inflammatory responses and PAI-1 expression, thereby disrupting fibrinolytic capacity (Hassanabad *et al.*, 2021). Nearly all patients undergoing intra-abdominal surgery develop peritoneal adhesions, impacting the quality of life of millions worldwide due to its related complications, such as bowel obstruction, relaparotomy, chronic abdominal and pelvic pain, and infertility (Moris *et al.*, 2017). Given the inevitability of post-abdominal surgery adhesion formation, preventive measures are crucial for reducing the economic burden associated with adhesions.

Intraperitoneal lavage, particularly with normal saline, is a common preventive strategy to separate peritoneal surfaces temporarily, allowing healing and preventing adhesion formation. However, non-physiological properties in normal saline, including its osmolality and acidic pH, can paradoxically increase the risk of adhesions by promoting mesothelial cell dysfunction and inflammatory responses, thus impairing the fibrinolysis essential for preventing adhesion formation (Cwalinski *et al.*, 2016). Recent studies have highlighted the potential benefits of using alternative lavage solutions like sterile water, which, due to its hypotonic nature, may reduce adhesions by preventing neovascularization and facilitating fibrinolysis (Octaviani *et al.*, 2017). Although the concept of peritoneal cavity lavage was first proposed in 1905, the clinical effectiveness of lavage remains controversial. Routine use of normal saline for intraperitoneal lavage has been shown to increase the risk of adhesions, and to date, there is no standard solution type for peritoneal cavity lavage (Nunes, 2014).

This suggests a need to re-evaluate the standard practice with normal saline for intraperitoneal lavage and explore other options to mitigate the risk of postsurgical adhesions better. This study aimed to compare the effects of peritoneal lavage with normal versus sterile water on IL-1 levels and intraperitoneal adhesion following laparotomy in rats.

2. Materials and Methods

Study design

Using a post-test-only control group approach, this experimental study was conducted at the Integrated Biomedical Laboratory of the Faculty of Medicine, Udayana University, from June 2023 to July 2023. Twenty male Wistar rats aged 2-3 months weighing 250-300 g, considering a potential 20% dropout rate. The independent variables were the types of lavage solutions used. In contrast, dependent variables included IL-1 levels and the incidence of adhesions, with age, body

weight, and lavage solution temperature serving as control variables.

Chemicals

Research materials included intestine samples, normothermic normal saline, and normothermic sterile water. The equipment used included digital scales for weighing (0.1 to 10 kg range), sterile minor sets for performing laparotomies, thermometers for measuring lavage solution temperature, and laboratory examination tools for assessing IL-1 levels via ELISA and macroscopically evaluating adhesion grade.

Animal Study and Surgical Procedure

All experimental animals were observed for one week to allow for adaptation to the food and environment and to ensure their health. In the second week, the rats provided by the animal testing laboratory were randomly divided into two groups of ten each. All rats were housed in iron cages, 2-3 per cage, with free access to food and water under standard laboratory conditions (room temperature at 21°C and a 12-hour light/dark cycle). The rats were fasted for 12 hours before surgery but still had free access to water. Anaesthesia was administered intramuscularly using ketamine at a dose of 5-10 mg/kg bw. Surgery was performed aseptically using sterile instruments. The anterior abdominal wall was shaved with a razor and disinfected with 10% povidone-iodine. Under semi-sterile conditions, a 3-cm midline incision was made, deepening to penetrate the peritoneum. The peritoneum was then opened, and an abrasion was performed on the caecum area until bleeding spots appeared, followed by intra-abdominal lavage using 5 ml of normothermic normal saline in the control group and 5 ml of normothermic sterile water in the treatment group. After lavage, the peritoneum was not sutured; instead, the fascia and skin were closed using nonabsorbable multifilament 3.0. Seven days later, a relaparotomy with a "U" shaped incision was made at the site of the previous surgery in each group to assess adhesion macroscopically.

Evaluation of Interleukin-1 (IL-1) and the incidence of adhesions

This analysis was conducted to determine the effects of intra-peritoneal lavage using normal saline and sterile water on IL-1 levels and the incidence of adhesions in male Wistar rats following laparotomy. Intestinal samples were taken from all experimental rats for IL-1 levels assay using the ELISA method. Subsequently, all rats were euthanized using a high dose of sodium thiopental (200 mg/kg bw), and their carcasses were disposed of in an incinerator. The

grade of intraperitoneal adhesion was measured using Zühlke's classification.

Data Analysis

The impact of peritoneal lavage on IL-1 levels was statistically tested using the unpaired T-test for normally distributed data. Meanwhile, the effects of peritoneal lavage on adhesion occurrence were statistically tested using the Chi-Square test. The magnitude of the effect was expressed as an odds ratio (OR), with significance defined as $p < 0.05$ with a 95% confidence interval.

3. Results

Effect of Lavage Solution on the IL-1 Level and Grade of Adhesion

The mean IL-1 level in the rats that received normal saline was 57,456 pg/ml, while the mean IL-1 level in the rats that received sterile saline was 37,111 pg/ml (Table 1). The unpaired T-test showed a significant difference ($p < 0.001$) in IL-1 levels between the two groups, with lower IL-1 levels observed in the sterile water group compared to the saline group.

Four rats in normal saline experienced grade 4 adhesions, five had grade 3 adhesions, and one had grade 2 adhesions, while in the group treated with sterile water lavage, two rats experienced grade 3 adhesions, five had grade 2 adhesions, and three had grade 1 adhesions (Table 2). A significant difference ($p = 0.014$) was found in the adhesion grade between the groups, with grades 1 and 2 being more prevalent in the sterile water group. In contrast, grade 3 and 4 adhesions were more common in the normal saline group. The grade of adhesion for each lavage solution group is illustrated in Figures 1 and 2.

Relationship between IL-1 Levels and Adhesion

The Chi-Square test showed a significant relationship between IL-1 levels and adhesion incidence

Table 1. IL-1 levels in the rats received normal saline and sterile water

Lavage Solution	IL-1 levels (pg/mL)	P value
Normal saline	57, 456.3 ± 10,583.41	<0.001*
Sterile Water	37,111.6 ± 6,535.61	

Table 2. Effect of Lavage Solution on the Grade of Adhesion

Lavage Solution	Zühlke's Classification				Total	P value
	Grade 1	Grade 2	Grade 3	Grade 4		
Sterile water	3	5	2	0	10	0.014*
Normal saline	0	1	5	4	10	
Total	3	4	7	4	20	



Figure 1. Grade 3 adhesions in the normal saline group, showing the presence of adhesions between the cecum and colon as well as adhesions between the intestines

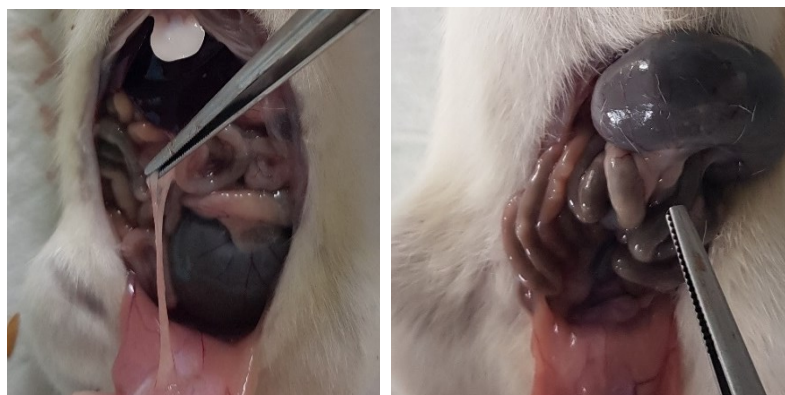


Figure 2. Grade 1 adhesions in the sterile water group, showing adhesions between the small intestine and the abdominal wall (left) and adhesions between the cecum and colon (right)

for each grade of adhesion (Table 3).

Further analysis was conducted to determine the IL-1 level cut-off for adhesion incidence, and the samples were divided into mild-strong adhesion (grade 1-2) and strong-very strong adhesion (grade 3-4). The ROC curve, shown in Figure 3, had an area under the curve (AUC) of 0.869, indicating high accuracy. The optimal cut-off was determined using the Youden index. In this study, an IL-1 level cut-off of 44,878 pg/mL showed 81.8% sensitivity and 88.9% specificity for grade 3 and 4 adhesions. This cut-off level was significantly associated ($p = 0.005$) with the incidence of grade 3 and 4 adhesions (OR 36; 95% CI 2.72–476.28), indicating that an IL-1 level of 44,878 pg/mL is a 36-fold risk factor for grade 3 and 4 adhesions.

4. Discussion

This study compared the effects of intra-peritoneal lavage using normal saline and sterile water on IL-1 levels and peritoneal adhesions following laparotomy in male Wistar rats. Our study's findings indicate that intraperitoneal lavage with sterile water results in significantly lower interleukin-1 (IL-1) levels compared to 0.9% NaCl, pointing towards a less inflammatory and adhesion-promoting environment post-laparotomy in Wistar rats. This research contributes to the existing body of knowledge by explicitly quantifying the inflammatory response and adhesion formation associated with these two commonly used lavage solutions. Reducing IL-1 levels and subsequent adhesion severity with sterile water use is a critical insight, offering a novel approach to potentially mitigating one of the most prevalent postoperative complications.

Following surgeries like laparotomy or laparoscopy, peritoneal trauma initiates hypoxia and inflammation, leading to oxidative stress and the generation of reactive oxygen species (ROS). This process results in peritoneal fibrosis and intraperitoneal adhesions (Tabibian *et al.*, 2017). Inflammation and a disrupted balance between coagulation and fibrinolysis, inhibiting fibrin breakdown, are key in adhesion pathogenesis. Consequently, fibroblast invasion and extracellular matrix production occur, forming adhesions between abdominal organs or cavity walls in 63–97% of post-major abdominal surgery cases (Arung *et al.*, 2011; Fortin *et al.*, 2015). These adhesions can lead to complications such as chronic abdominal pain, intestinal obstruction (or small bowel obstruction - SBO), and infertility. Typically, obstructions from adhesions are due to kinking, angulation, or compressive tissue bands, with the potential for strangulation affecting intestinal circulation. Adhesions are linked to a >20% readmission rate within a year post-surgery, contributing to 1–3% of surgical readmission cases in the USA

Table 3. Relationship between IL-1 Levels and Adhesion Incidence

Adhesion grade	Average IL-1 levels (pg/mL)	P value
Grade 1	30,128 ± 5,060.79	0.008*
Grade 2	44,599.83 ± 13,085.68	
Grade 3	48,606 ± 8,009.41	
Grade 4	61,863 ± 11,270.06	

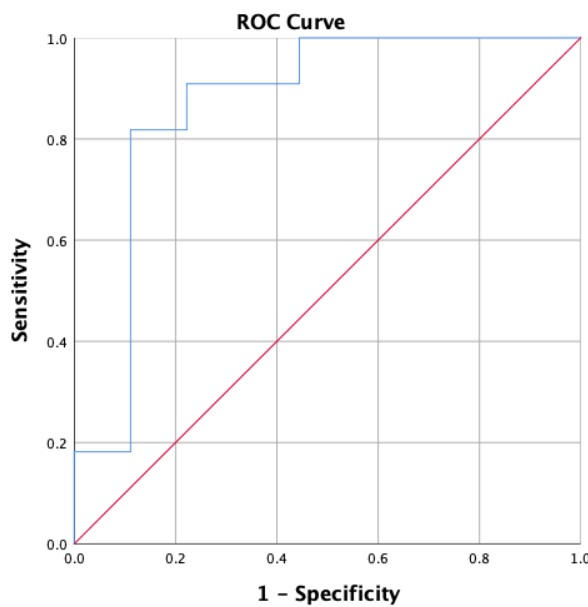


Figure 3. ROC curve of IL-1 against grade 3 and 4 adhesion levels

(Moris *et al.*, 2017).

To our knowledge, no study has investigated the effect of different solutions used for intraperitoneal lavage on IL-1 levels. A survey by Cheong *et al.* demonstrated higher IL-1 levels in patients with severe adhesions in the pelvic organs (Cheong *et al.*, 2002a). In addition, a clinical trial showed that IL-1 levels were significantly higher after 48 hours in patients with postoperative adhesions (Cheong *et al.*, 2002b). A study demonstrated the correlation between IL-1 levels and adhesion in a rat, showing that IL-1 injection into the peritoneum increased the risk of postoperative adhesion formation (Hershlag *et al.*, 1991). Conversely, administering IL-1 antibodies in the pelvic cavity reduced adhesions in rats (Kaidi *et al.*, 1995). This aligns with the finding from our study showing that high IL-1 levels (with a cut-off of 44.878 pg/mL) were associated with a 36-fold higher risk of developing grade 3 and 4 adhesions. Therefore, high IL-1 levels in intraperitoneal lavage with normal saline may indicate that physiologically, the risk of inflammation-mediated adhesion is higher with normal saline solution compared to sterile water.

Our study also directly compared adhesion levels after administering different lavage solutions. It

demonstrated a significant difference in intraperitoneal adhesion levels between sterile water and normal saline, where sterile water, with its H₂O content, was associated with milder degrees of adhesion. This result is like the research by Octaviani *et al.* (2017), which showed that the use of sterile water for intraperitoneal lavage was associated with significantly lower degrees of adhesion ($p = 0.047$) compared to the use of 0.9% NaCl in white rats. Intraperitoneal lavage aims to aid the peritoneum's defense mechanism against contaminants that can trigger infections, such as pathogens and tissue debris (blood, bile, etc.). Crystalloid solutions like 0.9% NaCl have been commonly used as lavage solutions, working as a mechanical barrier on the peritoneum surface and facilitating bacterial mass dilution (Garg *et al.*, 2013). The lower adhesion rate with sterile water in this study may be due to its hypotonic structure, which is nonpyrogenic and nonionic. Hypotonic solutions have a lower solute concentration, allowing water to move between cells and causing cell swelling. The condition of cell swelling can prevent neovascularization and the formation of fibrous tissue in intraperitoneal adhesions (Octaviani *et al.*, 2017; Halperin *et al.*, 2010).

The pathophysiology of intraperitoneal adhesion using 0.9% NaCl has been explored in prior research, highlighting the risk of adhesion formation with this lavage solution. In vitro studies by Winckiewicz *et al.* (2007) demonstrated that 0.9% NaCl is associated with an imbalance between tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1), where a decrease in tPA leads to fibrin deposits. Similarly, research by Cwalinski *et al.* (2015) found that using 0.9% NaCl is related to intraperitoneal adhesion events through increased tissue factor (TF) release and decreased tPA. These molecules are involved in the coagulation cascade for fibrin formation in intraperitoneal adhesions. Moreover, as an isotonic solution, 0.9% NaCl has the same solute concentration as an intracellular solution, and its use in intraperitoneal lavage does not induce cell membrane molecule movement. Hence, no cell swelling occurs. This lack of cell swelling allows angiogenesis and neovascularization processes to persist, forming adhesion (Octaviani *et al.*, 2017). Both in vivo and in vitro studies have shown the pathophysiology of peritoneal damage with 0.9% NaCl usage. It has been reported to decrease viability and fibrinolytic activity in peritoneal mesothelial cells, increasing the risk of adhesion formation (Brebrowicz *et al.*, 2005). Polubinska *et al.* (2008) also reported that, aside from its profibrotic effects, 0.9% NaCl could increase Reactive Oxygen Species (ROS) formation, detected through fluorescent probes. This ROS increase is suspected to be mediated by enhanced sodium influx into the

cytosol through the Na(+)-H(+)-ATPase transport system during 0.9% NaCl solution administration. The resulting oxidative stress and changes in extracellular osmolarity disrupt fibrinolytic activity, culminating in intraperitoneal adhesion formation (Polubinska *et al.*, 2008).

Interestingly, Sortini *et al.* (2006) reported different outcomes, where 0.9% NaCl usage showed a lower risk of adhesion with higher survival rates in rats than distilled water. However, this study's subjects were injected with lethal *Escherichia coli* before irrigation, which could affect the outcomes. Another clinical outcome was reported by Shiozaki *et al.* (2014), who compared cancer nodule formation in the peritoneum in an in vivo gastric cancer cell model. They found that distilled water injection into the peritoneum was associated with lower dissemination nodules than incubation with isotonic 0.9% NaCl solution. Distilled water's hypotonic shock effect, which also causes cell swelling, was observed in both in vivo and in vitro cancer cell studies (Hirotaka *et al.*, 2014). It is important to note that the relationship between 0.9% NaCl and adhesions in intraperitoneal lavage may be influenced by temperature. A study by Koca *et al.* (2016) showed that administering normothermic (37°C) 0.9% NaCl could cause higher PAI-1 inhibition compared to hypothermic (21°C) solution, leading to a reduction in postoperative adhesion events. The underlying mechanism might relate to hypoxia and acidosis occurring at hypothermic temperatures, which can induce coagulopathy and decrease fibrinolytic activity. Previous research indicated increased adhesion formation with 0.9% NaCl irrigation at temperatures above 37°C, likely related to the inflammatory processes that can occur at higher temperatures (Molinas *et al.*, 2010).

The study's strengths lie in its controlled design, allowing for a clear comparison between the effects of NaCl 0.9% and sterile water on IL-1 levels and adhesion formation. However, it is not without limitations. The use of an animal model, while valuable, necessitates cautious extrapolation to human surgery. Variability in surgical technique, patient characteristics, and postoperative care could influence the generalizability of our results. Moreover, the study's relatively short duration precludes insights into long-term adhesion outcomes and potential complications from using sterile water in humans.

5. Conclusions

Peritoneal lavage with sterile water was associated with lower postoperative adhesion degrees and postoperative IL-1 levels compared to Future studies should also explore the long-term effects of using various solutions for intraperitoneal lavage and

compare adhesion levels among other lavage solutions.

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Conflict of interest

All authors have no conflict of interest in this article.

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