Immunopathologia Persa
http immunopathol.com
DOI:10.34172/ipp.2025.43798

Human amniotic membrane versus topical simvastatin solution in reducing intestinal adhesion after laparotomy in rats

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Received 25 Nov. 2024
Revised: 4 Jan. 2025
Accepted 14 Jan. 2025
ePublished 26 Jan. 2025

Keywords: Intra-abdominal adhesions, Laparotomy, Simvastatin, Amniotic membrane, Postoperative complications

Abstract

Introduction: Intra-abdominal adhesions are common complications that follow laparotomy are common, prompting surgeons to strive for their reduction. Simvastatin has been promising due to its fibrinolytic and anti-inflammatory properties, but human amniotic membrane (HAM) is a promising alternative option.
Objectives: The current study compares the effects of amniotic membrane and topical simvastatin solution in reducing intestinal adhesions after laparotomy in rats.
Materials and Methods: Thirty Wistar albino rats were divided into three groups (HAM, simvastatin and placebo). The rats underwent midline laparotomy, and optimal adhesions were induced using the meso-stitch approximation method. Before closing the abdomen, in group one, disinfected human amniotic membranes were used to cover the viscera. In the second and third groups, simvastatin solution and distilled water were deposited into the abdominal cavity, respectively. Two weeks later, a second laparotomy was performed to compare the degree of adhesion, using the Hoffmann quantitative and qualitative Lauder questionnaire and histological report. The data were analyzed using SPSS software.
Results: The chi-square analysis was utilized for the clinical evaluation of adhesion severity across the three groups. The results showed a statistically significant difference between the three groups ($P<0.0001$). Statistical analysis to compare Lauder and Hoffman scores between the three groups showed that the degree of adhesion in simvastatin, HAM, and placebo groups had a statistically significant difference ($P<0.0001$).
Conclusion: Our findings revealed that preventive human amniotic membrane for postoperative adhesion formation in rats resulted in better surgical and histological outcomes than simvastatin or placebo.

Citation: Derakhshan R, Golmohamadi Khamene A, Kazeminejad B, Bayat M, Baghsheikhi H, Abbasi M, Sabet B. Human amniotic membrane versus topical simvastatin solution in reducing intestinal adhesion after laparotomy in rats. Immunopathol Persa. 2025;11(2):e43798. DOI:10.34172/ipp.2025.43798.

Introduction

Intra-abdominal adhesions occur in up to 94% of patients after undergoing laparotomy (1). Individual characteristics and underlying health conditions, such as diabetes or infections, increase the risk of adhesion formation (2). These adhesions are the leading cause of small bowel obstruction in industrialized countries (3), and approximately 30% of patients undergoing adhesive ligation surgery need necessary corrective operations. Therefore, prevention of this complication is crucial due to the high prevalence of adhesion complications and the lack of definitive treatment despite reoperation and new advances in surgical techniques (4, 5).

In recent years, researchers have explored various effective methods to prevent post-surgery adhesions, including anti-inflammatory agents, fibrinolytic agents, antibiotics, and mechanical barriers. (5). Intra-abdominal use of simvastatin, a fibrinolytic agent, has reduced intra-abdominal adhesions following laparotomy in rats (6). Despite promising results in animal studies, such agents have not demonstrated similar efficiency in humans, and no ideal approach exists (7).

Key point

Intra-abdominal adhesions are a common complication after laparotomy. This study in rats compared human amniotic membrane, simvastatin, and a placebo in preventing adhesions. This study, human amniotic membrane showed significantly better results than both simvastatin and placebo in reducing adhesion formation, based on both macroscopic and histological evaluation.

The potential application of amniotic derivatives has been considered due to their properties, such as promoting epithelialization and reducing inflammation and fibrosis (8). In some animal studies, amniotic membrane has successfully reduced postoperative adhesion formation (9-11). Other applications of amniotic membrane have been acknowledged in increasing the success of anastomosis healing and repairing recto-vaginal fistula in the animal model (12,13).

Objectives

This study investigated the effectiveness of prophylactic dry amniotic membrane and topical simvastatin solution on intestinal adhesion after laparotomy in rats.

Materials and Methods

Study design

This experimental interventional study was done on Wistar albino male rats weighing 250 to 300 g at Shahid Beheshti medical university laboratory center. All rats were housed in standard cages with specialized food and water for one week, maintaining identical conditions. The rats were randomly divided into three groups: Group one (disinfected human amniotic membrane [HAM]), group two (simvastatin solution), and group three (control group with distilled water). According to the animal studies protocol, in the first group, rats were anesthetized intramuscularly with ketamine at 60 mg/kg/d. The rats were then subjected to a midline laparotomy with a 4 cm incision. Powderless gloves were used in all three groups during surgery.

To enhance adhesion, the meso-stitch approximation method, known as the optimized peritoneal adhesion model, was utilized following the study by Poehnert et al (14). The viscera in the first group were covered with a dry, disinfected amniotic layer prepared by the Royan institute. In the second and third groups, before closing the abdomen, 2 cc of simvastatin solution at a dose of 40 mg/kg and 2 cc of distilled water were deposited into the abdominal cavity, respectively. Subsequently, the abdominal wall layers were repaired with Prolene mesh and closed with nylon (0-3) in all three groups. Finally, the skin was repaired with nylon (0-3).

After two weeks, all 30 rats underwent reoperation. The rats were anesthetized with ketamine and then euthanized with chloroform gas. Laparotomy was performed again, and the degree of adhesion was compared in the three groups. Adhesions were scored using (a) Observation during operation with quantitative Hoffman questionnaire (15), (b) Observation during operation with qualitative Lauder questionnaire (16), and (c) Histologic examination was conducted with Nair score (17).

Statistical analysis

After collecting the required information, the data was analyzed using SPSS software (version 24) to assess qualitative and quantitative variables, including frequency, mean, and standard deviation. One-way ANOVA and chi-square tests were conducted for two-way analysis. In all the cases mentioned, a significance level of less than 0.05 was established for the *P* value.

Results

This study included 30 male rats in three groups of amniotic membrane, simvastatin, and placebo-treated (distilled water) rats. No infections were observed in the groups after the first surgery, and no rats died during the two weeks leading up to the second operation. This study classified adhesion based on severity, including no adhesion, mild, moderate, and severe categories. The severity of adhesion was clinically evaluated in the three groups using chi-square analysis. The results indicated a statistically significant difference among the three groups ($P<0.0001$).

Statistical analysis to compare Lauder and Hoffman scores between the three study groups was performed by one-way ANOVA statistical test, which showed that the degree of adhesion in the groups of simvastatin, amniotic membrane, and placebo (distilled water) had a statistically significant difference ($P<0.0001$). Amniotic membrane group showed significantly less adhesion than the simvastatin group ($P<0.0001$) using both Lauder and Hoffmann criteria. The findings are presented in Figures 1 and 2.

The comparison of the frequency and severity of adhesions among the three study groups—clinically and qualitatively—demonstrated that the amnion membrane was significantly more effective than simvastatin ($P<0.001$). Furthermore, the severity of adhesions in the simvastatin group was lower than in the placebo group ($P=0.001$). On pathologic examination, amnion groups had better results than others (Table 1).

Discussion

The current study demonstrated that using HAM to prevent intra-abdominal adhesion formation resulted in

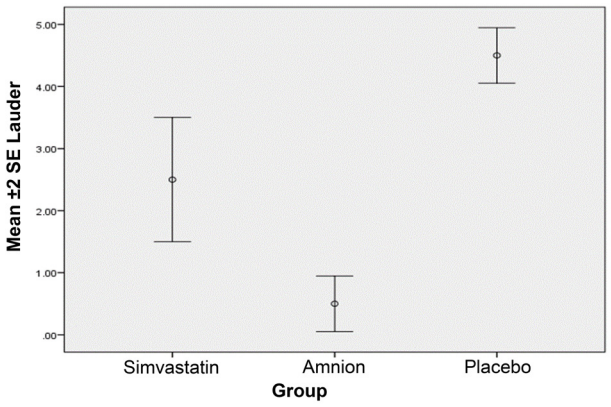

Figure 1. Comparison and dispersion of adhesion in three study groups based on Lauder criteria.

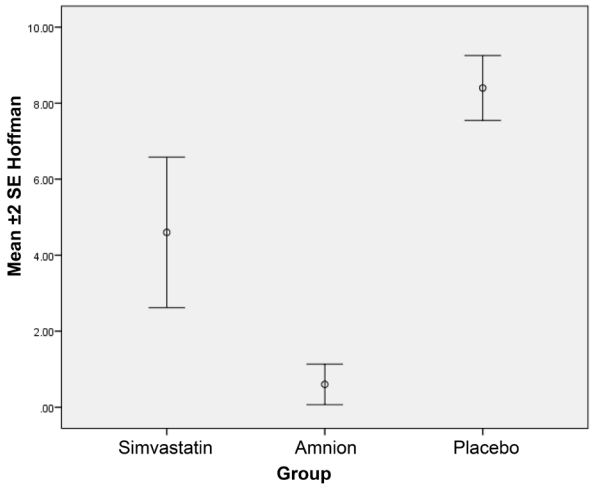

Figure 1. Comparison and dispersion of adhesion in three study groups based on Hoffman criteria.

better outcomes than simvastatin and placebo following laparotomy in rats. Lauder's and Hoffman's scores showed that the degree of adhesion significantly differed between the three groups. There was also a statistical difference in the two-way analysis of the data.

Intra-abdominal adhesions, usually presented as bands after abdominal surgery, are among the most important causes of intestinal obstruction (17,18) and are considered lifelong complications. Hitherto, only the barrier method for separating traumatized surfaces has effectively prevented adhesion formation in humans (5,19). HAM has many properties such as immune privilege possesses factors that regulate immune responses, prevention of scar tissue formation, rapid attachment to the wound surface, facilitating cell differentiation and attachment, induction and facilitation of cell migration, usability on all body surfaces, reducing inflammation and pain in the area, and homogeneity in tissue regrowth, which has shown promising results in animal studies.

To date, several studies have shown the effectiveness of HAM in preventing adhesion, healing skin wounds, and repairing recto-vaginal fistulas and duodenal perforations (11,13,20,21). There have been few studies on the effects of HAM in preventing post-laparotomy adhesion, and these studies have produced varied results. Studies by Ryan et al (23) and Longaker et al (24) reported no significant differences in adhesion formation and the level of adhesion when comparing the HAM group to other groups. In our study, using a HAM prevented adhesion formation compared to simvastatin and placebo (distilled water), consistent with the results of several similar studies (9,10,22).

Ryan et al (23) hypothesized that blood products are a source of fibrin formation. Hyaluronic acid is a plentiful glycosaminoglycan found in amniotic fluid. Hyaluronic acid is a contributing factor to fetal wound healing and, therefore, seems to play a role in the prevention of intrauterine adhesion formation (24). Although this substance is present in adults' wounds, it is produced continuously and in considerable amounts only in fetal wounds. Increasing the level of hyaluronic acid in the wound, on the one hand, leads to the improvement of wound healing due to the proper organization of collagens; on the other hand, it can result in decreased adhesion formation (25). Choi et al (26) reported the effectiveness of HAM in reducing postoperative adhesions after laminectomy in rats, which confirms the above hypotheses. The present study also showed that simvastatin significantly reduced the adhesion intensity compared to placebo (distilled water). Simvastatin suppresses transforming growth factor-β (TGF-β) and TGF-β-induced connective tissue growth factor production in a concentration-dependent manner. This immunomodulatory function might be the reason for the reported outcome (27). Moreover, a previous study of this subject showed that oral and topical use of simvastatin reduces postoperative adhesions in rats (28).

Conclusion

The present study showed that human amniotic membrane and topical simvastatin reduce the adhesion induced by laparotomy in the animal model. In addition, no cases of human amniotic membrane infection occurred in the present study. Similar studies on a larger sample size in the laboratory are needed to validate our results before they can be studied further in human clinical trials.

Acknowledgments

The authors would like to acknowledge of Shahid Beheshti University of Medical Sciences

Authors' contribution

Conceptualization: Babak Sabet.
Data curation: Amin Golmohamadi Khamene, Maryam Abbasi.
Formal analysis: Babak Sabet.

Table 1. Comparison of adhesion in three study groups based on Nair score

Under-study variable	Simvastatin group No. (%)	Amniotic membrane group No. (%)	Placebo-treated (distilled water) group No. (%)	*P* value
Without adhesion	2 (20)	6 (60)	-	<0.0001
Fat	2 (20)	4 (40)	-	
Fat and fibrosis	5 (50)	-	4 (40)	
Fibrosis	1 (10)	-	6 (60)	

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Conflicts of interest

There are no competing interests.

Ethical issues

The researchers adhered to the ethical guidelines for working with laboratory animals. The current study received approval from the Faculty of Medicine at Shahid Beheshti University of Medical Sciences under (Ethical code #IR.SBMU.MSP.REC.1400.695). Besides, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the author.

Funding/Support

None.

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