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Original research

Comparison of lovastatin and hyaluronic acid/carboxymethyl cellulose on experimental created peritoneal adhesion model in rats

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ABSTRACT

Objectives: The aim of this experimental study was to compare the effectiveness and reliability of lovastatin and hyaluronic acid + carboxymethyl cellulose (Seprafilm).
Materials and methods: Thirty two female Wistar–Albino rats weighing between 250 and 300 g were used in the study. The rats were divided into four groups as sham, control, lovastatin and Seprafilm each of which contained 8 rats. All rats were sacrificed on the 14th day after surgery. Macroscopic adhesion, microscopic adhesion and tPA, MDA and NO values were evaluated.
Results: Macroscopic adhesion formation was significantly lower in the sham and study groups than in the control group ($p < 0.05$). Microscopic classification adhesion formation was significantly lower in the sham and study groups than in the control group ($p < 0.05$), and the tPA, MDA and NO values showed statistically significant differences among the groups.
Conclusion: Lovastatin and Seprafilm were equally effective in preventing postoperative intra abdominal adhesions. The study groups were showed significant superiority to the control group.

1. Introduction

Postoperative intra abdominal adhesions are the most frequent complication of surgery, although often not recognized as such. After abdominal surgery, 67–93% of patients develop adhesions.[1,2] Between 0.3 and 10.7% of patients develop intestinal obstruction after intra-abdominal surgery.[3] Other consequences of intra abdominal adhesions may cause infertility (15–20%), chronic pelvic pain, dyspareunia and ectopic pregnancy. In addition difficult reoperative surgery, increase in bleeding, injury to adjacent organs and such effects may occur.[3,4] The economic burden of adhesion-related hospital readmissions and reoperations is enormous.[5,6] The pathophysiology of adhesion formation has been researched widely. This process is activated by tissue factor or more specifically by the fibrin gel matrix.[7] Fibrinogen, is a soluble protein and is located between the tissues and blood products.

It reacts with thrombin and forms fibrin monomers and fibrinogen is polymerized. Initially the fibrin polymers are soluble and reveal injured surfaces during surgery. If they stay in contact with coagulation factors such as Factor XIIIa for a long time, the fibrin polymers forma fibrin-gel matrix.[8]

Under normal circumstances, this fibrogenesis is in balance with fibrinolysis. The process of fibrinolysis is driven by the enzyme plasmin, which is derived from its inactive substrate plasminogen by tissue-type plasminogen activator (tPA). In turn, tPA is inhibited in its reaction by plasminogen activator inhibitor-1 (PAI-1), to maintain a balance. In the abdominal cavity, tPA is responsible for 95% of plasminogen conversion.[7] Intra-abdominal surgery disturbs the balance between tPA and PAI-1resulting in decreased fibrinolytic activity and an increase in fibrin exudate, eventually leading to an increase in adhesion formation.[9]

Sodium hyaluronate/carboxymethyl cellulose(HA/CMC)(Seprafilm) is a bioreabsorbable membrane which reduces the formation of adhesions. This membrane is transformed into a gel within 24 h after application. During the recovery phase of peritoneal adhesion it prevents the formation of damaged tissues by providing physical separation. All traces of the gel are excreted from the body within 28 days.[10,11] Clinical and experimental studies have shown that Seprafilm prevented intra-abdominal adhesions.[12–14]

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Statins increase extracellular fibrinolytic activity, decrease expression of tissue factor and platelet activation and can cause a major reduction in the risk of stroke and cerebral ischemia.

Because of this fibrinolytic activity of statins, suggested their possible use in the treatment of intra-abdominal postoperative adhesions. In an experimental adhesion formation model Aorans and et al. found that intraperitoneal application of lovastatin and atorvastatin in rats reduced postoperative adhesion.[15]

In our study, we aimed to evaluate the effects of lovastatin and to compare it with hyaluronic acid + carboxy methyl cellulose which has previously been proved to be effective in many clinical and experimental studies.

2. Materials and methods

2.1. Animals and preoperative preparation

This experimental study was performed at Erciyes University's Hakan Cetinsaya Experimental and Clinical Research Center in 2008. The study was approved by the Ethics Committee (Date:21.11.2007, number:07/11). Thirty two female Wistar–Albino rats weighing between 250 and 300 g were used in the study. All rats were fed ad libitum with standard rat chow and tap water. All subjects were kept in 12 h darkness and 12 h light for before and after study (at a standard temperature of −22 °C). The subjects were kept under observation at least 48 h before they were included in the study. After 12 h of starvation, the subjects were weighed and divided randomly into four groups as sham, control and two study groups each containing 8 rats.

Group 1 (sham):After laparotomy the caecum, ileum and right corner of the uterus was palpated and the abdomen was closed.

Group 2 (control): The experimental model was performed in this group of rats and before closing the abdomen 5 cc saline was administered intraperitoneally.

Group 3 (lovastatin): The experimental model was performed in this group of rats and before closing the abdomen 30 mg/kg lovastatin was administered intraperitoneally (Sigma M2147, mevinolin from Aspergillus sp).

Group 4 (Seprafilm): The experimental model was performed in this group of rats and before closing the abdomen 30 × 20 mm Seprafilm was applied intraperitoneally. (HA + CMC- Seprafilm-Genzyme Corporation, Cambridge, MA, USA).

2.2. Operative technique

Fifty mg/kg ketamine -HCL(Ketalar, Pfizer, Turkey) was administered intraperitoneally as an anesthetic agent. After administering the anesthetic agent the rats' abdomens were shaved with a shaver and stained with povidone-iodine. A 3-cm midline laparotomy incision was performed under sterile conditions. In this study the caecum, ileum and right corner of the uterus were abreded and bleeding points were created with the help of a lancet and only serosal injury was created by the surgeon blinded to group allocation. After this operation saline solution was administered to the peritoneal cavity of rats in the control group, while Seprafilm and lovastatin were administered intraperitoneally in the study groups. The peritoneal fascia was closed with 4/0 PDS and the skin with 3/0 silk at the end of the operation. All rats were returned to their cages after the operation and kept at an ambient temperature of 22 °C. They were fed with standard rat diet after surgery. In the control group one rat died during the study on the first postoperative day and a new rat was added. All rats were sacrificed with a high dose of anesthetic on 14th day after surgery. After being sacrificed, a paramedian laparotomy was performed immediately; the ceacum and abdominal side wall were evaluated for adhesion and 2 g tissue was taken from the adhesion region.

2.3. Macroscopic evaluation

Macroscopic evaluation of the intraperitoneal cavity was performed by a surgeon blinded to group allocation. Therefore, the data analysis was performed in a blinded fashion.

Majuzi classification is used for the evaluation of postoperative intraperitoneal adhesions.[16] According to the classification the following grades are used: Grade 0: no adhesion, Grade 1: very little and irregular adhesion, Grade 2: easily separable medium intensity adhesion, Grade 3: intense, not easily separable regular adhesion, Grade 4: very hard, not easily separable and homogeneous adhesion.

2.4. Histopathologic evaluation

After macroscopic evaluation, samples were taken from the fibrous bands between the caecum and the peritoneum and a histopathological examination was performed by a pathologist blinded to the groups. Tissue samples were fixed for 12 h in a 10% buffered neutral formalin solution. After a routine follow-up, the samples were embedded in paraffin blocks and sections of 4–5 μm thickness were stained with hematoxylin and eosin (H&E) and.

These were finally examined under a light microscope. The Zühlke scoring system was used to assess the adhesions.[17] According to the scoring system the following grades were used 1: Loose connective tissue, cell-rich old and new fibrin, fine reticulin fibers 2: Connective tissue with cell and capillaries, few collogen fibers, 3: Connective tissue firmer, fewer cells, more vessels, few elastic and smooth muscle fibers 4: old firm granulation tissue, cell poor, serosal layers hardly distinguishable.

2.5. Measurement of nitric oxide, malondialdehyde

Nitric oxide (NO), malondialdehyde (MDA) and tissue plasminogen activator (tPA) parameters were examined in the biochemical evaluation.

For measurement of malondialdehyde (MDA),[18] 0.5 ml of serum was added to 2.5 ml of 20% TCA and then 1 ml of 0.67% thiobarbituric acid (TBA). The mixture was incubated at 100 °C for 30 min. After cooling, the sample was extracted with 4 ml *n*-butanol and centrifuged at 3000 rpm for 10 min. The absorbances of the extract were measured at 535 nm and the results were expressed as μmol/l, using the extinction coefficient of 1.56×10^5 l/mmol cm.

Nitrate/nitrite in serum was determined according to the Griess reaction.[19] A 100 μl measure of serum was incubated for 30 min at 37 °C in the presence of 0.2 U/ml nitrate reductase, 50 mmol/l Hepes buffer, 5 μmol/l FAD and 0.1 mmol/l NADPH in a total volume of 500 μl. Following incubation, 5 μl of lactic dehydrogenase (1500 U/ml) and 50 μl of 100 mmol/l pyruvic acid were added to oxidize any unreacted NADPH. The sample was then incubated for 10 min at 37 °C. One milliliter of premixed Griess reagent (equivolume of 0.2% naphthylethylenediamine and 2% sulfanilamide in 5% phosphoric acid) was then added. After 10-min incubation at room temperature, the absorbances of the samples were determined at 543 nm. Nitrate concentrations were calculated from the difference between the values obtained in the presence and absence of nitrate reductase. The results were expressed as μmol/l, using known concentrations of nitrate.

For measurement of tissue plasminogen activator (tPA) a rat tPA ELISA Kit (Innovative Research 21315 Hilltop, Southfield) was used. tPA binds to the capture antibody coated on the microtiter plate. Free, latent and complexed tPA will bind to the capture antibody. An

HRP detection system using TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of purified tPA.

2.6. Statistical analysis

Statistical analysis was performed by using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) for Windows Vista. Data are expressed as mean ± standard error (X ± SE) and median (min–max). Kruskal–Wallis and Mann–Whitney *U* tests were used for macroscopic and histopathological values, and the post-hoc Scheffe (ANOVA) test was used for a comparison of biochemical values. *P* values < 0.05 were considered to be statistically significant.

3. Results

3.1. Macroscopic evaluation

Four rats (50%) had no adhesions in the sham group. Seven rats in the control group (87%) had adhesions in different stages. Of these 25% were grade 3 adhesions. The total adhesion scores of the sham group, lovastatin group and Seprafilm group were significantly lower than the control groups ($p = 0.019$). There was no significant difference among the sham, Seprafilm and lovastatin groups. ($p = 0558$, $p = 0819$, $p = 0680$) (respectively) (Table 1 and Fig. 1).

3.2. Histopathological evaluation

Histopathological evaluation was performed according to the microscopic adhesion classification, where the thickness of connective tissue, reticulin and collagen fibers, cells and capillary vessels were taken into consideration. The total adhesion scores of the sham group, lovastatin group and Seprafilm group were significantly lower than the control groups ($p = 0.08$) ($p < 0.05$). There was no significant difference among the sham, Seprafilm and lovastatin groups. ($p = 0.28$, $p = 0.02$, $p = 0.28$) (respectively) (Table 1 and Fig. 2).

3.3. Biochemical evaluation

There was statistically significant difference in tPA, MDA and NO levels ($p = 0.015$, $p = 0.003$, $p = 0.026$)(respectively). MDA and NO levels were higher in the control group. There was a statistically significant difference among the control groups and the other groups. In our study, tPA values were higher in the lovastatin group. There was a statistically significant difference between the lovastatin group and the control group ($p = 0.048$) ($p < 0.05$). However, no significant difference was found when the lovastatin group was compared with the sham and Seprafilm groups (Table 2).

4. Discussion

Postoperative intra abdominal adhesions, cause serious morbidity like pelvic pain, infertility, bowel obstruction and ureteral obstruction.[4,20] These problems can cause serious morbidity and mortality and may require reoperation for some patients. In addition, previous studies have shown that intra abdominal adhesions prolong planned or emergency re-laparotomy time and exploration and increase iatrogenic bowel injury.[5,6] Some materials are used to reduce adhesions. Seprafilm has the most obvious effectiveness in these tested preparations. The effectiveness of this material has been shown in many experimental studies.[2,21]

Seprafilm was applied to patients undergoing ileostomy surgery and adhesions were evaluated when the ileostomy was closed. This prospective, randomized, multi-center study showed that Seprafilm reduced the amount of adhesions in patients.[22] Some rare complications have been reported due to Seprafilm, Remzi et al. reported three non-purulent peritonitis cases secondary to Seprafilm which necessitated relaparotomy, but mortality was not observed in the patients.[21]

HMG-CoA reductase inhibitors inhibit the rate-limiting enzyme in the production of cholesterol. This class of drugs (statins) has a well-studied lipid-lowering benefit; however, recent studies have revealed additional effect of statins beyond their impact on serum cholesterol levels. They have been shown to have potent anti-inflammatory, antioxidant and pro-fibrinolytic properties.[15,23,24]

Statins are used in the treatment of and the kidney, heart, lung, and skin fibrotic diseases for their antifibrotic effect owing to Rho kinase inhibition.[25–29]

The idea of using statins for intra abdominal postoperative adhesions was suggested because of their fibrinolytic activity. Aorans et al. found that intraperitoneal application of lovastatin and atorvastatin in rats reduced adhesion formation in an experimental model. In this experimental study the tPA levels increased 57%–379% and adhesion formation decreased 26%–58%.[15] Also, Burke et al. reported the antifibrotic effects of Simvastatin which acts inhibiting intestinal smad-3 phosphorylation.[30]

In our study, in the lovastatin group adhesion formation was significantly lower than in the control group. Lovastatin increases tissue type plasminogen activator (tPA) and decreases plasminogen activator inhibitor-1(PAI-1) production by human mesothelial cells. An increase in the tPA/PAI-1 ratio upregulates fibrinolysis against fibrinogenesis and fewer adhesions are formed.[31]

Free oxygen radicals play an important role in intraperitoneal adhesions which are caused by trauma, infection, or ischemia. Oxygen radicals affect the integrity of the cell membrane and also increase prostaglandin and thromboxane production by affecting the metabolism of arachidonic acid and contribute to adhesion formation. In this experimental study, NO and MDA were evaluated as indicators of oxidative stress. NO and MDA levels in the control group were significantly higher compared to the sham and study groups and it has been shown that free oxygen radicals are effective against adhesion formation.

Finally, we observed that lovastatin was as effective as Seprafim. MDA and NO levels in the control group were higher than the other groups. The highest tPA value was in the lovastatin group and there was a statistically significant difference with the control group ($p = 0.048$) ($p < 0.05$).

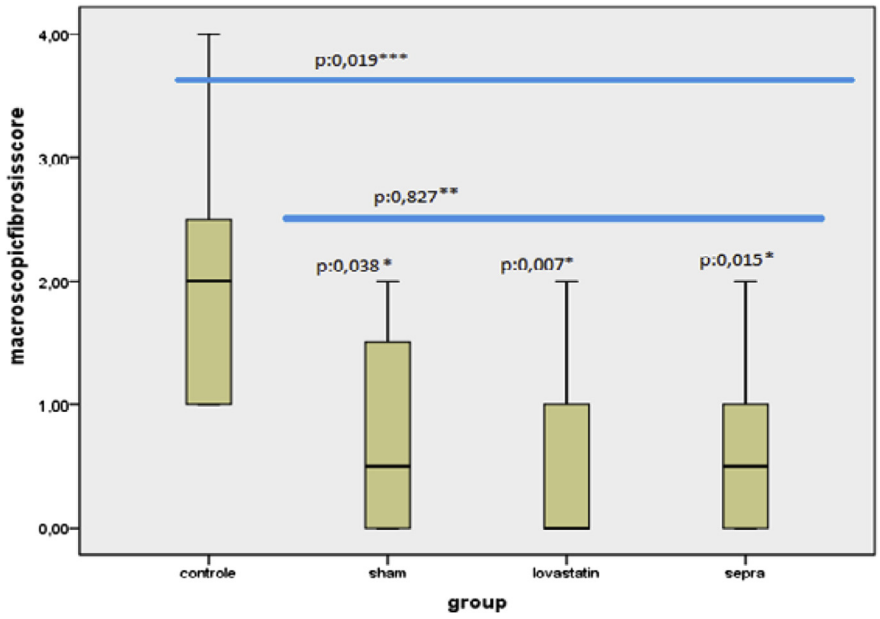
In the control group one rat died during the study on the first postoperative day and a new rat was added. None of the rats revealed wound infection or intra abdominal abscess.

When all these data are combined, it is seen that the anti-adhesion effect of lovastatin deserves more detailed investigation. Furthermore, we need to determine lovastatin's side effects and the

Table 1
Macroscopic and microscopic adhesion score values of the groups.

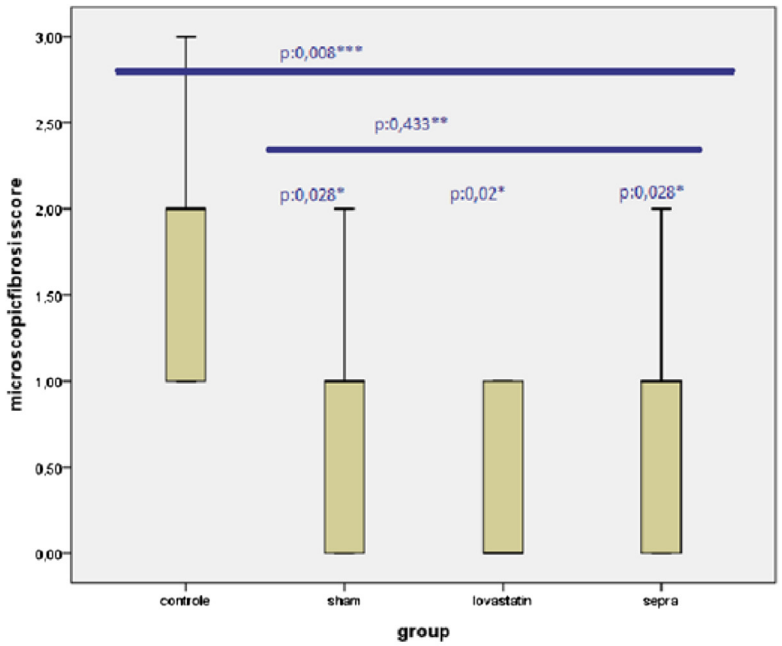
	Control	Sham	Lovastatin	Sepra	*P* value
Macroscopic fibrosis score (M ± SD)	2.0 ± 1.06	0.75 ± 0.88	0.5 ± 0.75	0.62 ± 0.74	0.019
Microscopic adhesion score (M ± SD	1.75 ± 0.70	0.75 ± 0.70	0.37 ± 0.51	0.75 ± 0.71	0.008

M:Mean, SD:Standart Deviation.



- * comparison of sham, lovastatin and sepra groups with controle group seperately
- ** within sham, lovastatin and sepra groups
- *** within all groups

Fig. 1. Comparison of macroscopic adhesion values in groups. * Comparison of sham, lovastatin and sepra groups with control group separately. ** Within sham, lovastatin and sepra groups. *** Within all groups.



- * comparison of sham, lovastatin and sepra groups with controle group seperately
- ** within sham ,lovastatin and sepra groups
- *** within all groups

Fig. 2. Comparison of microscopic adhesion values in groups. * Comparison of sham, lovastatin and sepra groups with control group separately. ** Within sham, lovastatin and sepra groups. *** Within all groups.

Table 2
tPA, NO and MDA values in groups.

	Control group	Sham group	Lovastatin group	Sepra group	*P* value
tPA values (M ± SD)	0.385 ± 0.478	0.818 ± 0.310	1.485 ± 0.319	0.843 ± 0.285	0.015
NO values (M ± SD)	0.877 ± 0.021	0.099 ± 0.019	0.083 ± 0.020	0.064 ± 0.015	0.003
MDA values (M ± SD)	1.915 ± 0.415	1.057 ± 0.088	0.755 ± 0.107	0.947 ± 0.084	0.026

M: Mean, SD: Standard Deviation.

effectiveness of the prevention of adhesion in human applications, and this will reveal exactly, whether or not it can be used as a real alternative to Seprafilm in clinical practice.

5. Conclusion

Lovastatin and Seprafilm are equally effective in preventing postoperative intra abdominal adhesions. The cost of lovastatin is lower than that of seprafilm and this is a notable advantage.

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