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Original Article

Effect of orally administered simvastatin on prevention of postoperative adhesion in rats

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Abstract: Aim: Formation of adhesions in the abdominal region appearing after abdominal pelvic surgery lead to infertility, chronic pelvic pain, intestinal obstructions, difficulty and morbidity at the following operations, and increased morbidity. The aim of our study is to examine the effectiveness of orally administered simvastatin on preventing the postoperative adhesion. Materials and methods: 20 male Wistar Albino rats weighing 230-250 gr were used. The rats were housed for 12 hours day and 12 hours night cycles in cages and were divided into two groups, namely study and control group. Microscopic evaluation of adhesion was assessed under 5 main topics which are the signs of inflammatory response; inflammation, activation, fibroblast activity, vascularity, presence of giant cell. Activation was scored as follows: (0) no activation, (1) while activation was accepted as present the score for other parameters was evaluated between 0 to 3 according to the increased severity. After evaluating all topics separately, the average of all scores has been assessed in both groups. Results: As a result of the macroscopic evaluation of postoperative intra-abdominal adhesions, the percentage of adhesion in simvastatin applied group was found to be 0.8 ± 0.17. This value was calculated as 0.6 ± 0.2 in the control group. Regarding the severity of adhesion, while in the simvastatin applied group the value was found to be 9.1 ± 4, in the control group it was 6.8 ± 3. The general adhesion score was found to be 7.7 ± 4.2 in simvastatin applied group and 5.1 ± 3.7 in control group. Conclusion: In this experimental study it was showed that orally administered simvastatin has no significant effect on preventing formation of postoperative adhesions.

Keywords: Surgery, adhesion, simvastatin

Introduction

The formation of intraabdominal adhesions following the abdomino-pelvic surgery associated with many problems including infertility, chronic pelvic pain, intestinal obstruction, and even mortality [1-5]. The incidence of small intestinal obstructions can be as high as 59% to 93% with postoperative adhesions [6-8]. The risk of recurrent intestinal obstruction with adhesion is not rare. Pathogenesis of the postoperative adhesion formation was investigated in various studies and several agents have been examined for prevention of adhesion formation [4, 5, 9]. Anti-inflammatory drugs, antibiotics, chemical and physical barriers failed to prevent adhesion formation [10-12]. In order to prevent adhesion formation, strategies including reduction of peritoneal damage, prevention of coagulation of serous exudate, removal of fibrin deposits, inhibition of fibroblast activities, prevention of collagen deposits and angiogenesis were evaluated.

In this experimental study the effect of orally administered simvastatin on prevention of intra-abdominal adhesions was investigated.

Materials and methods

This study was approved by the Ethical Committee of Experimental Animals in Istanbul University, Institute of Experimental Medical Research. In our study, totally 20 male Wistar Albino rats weighing 230-250 gr were used. The

Table 1. Comparison of adhesion characteristics between Group I and Group II

	Simvastatin (Group I)	Control (Group II)	*P* value
Adhesion severity	0. 8	0. 6	0.200
Adhesion rate	9. 1	6. 8	0.136
General adhesion score	7. 7	5. 1	0.141

rats were housed for 12 hours day and 12 hours night cycles in cages and were divided into two groups, namely study and control group. Group I as the study group was composed of 12 rats, Group II as the control group was composed of 8 rats. 48 hours prior to the operation Group I started to be applied 40 mg/kg dose 1x1 pulverized simvastatin tablet (Zocor®, Merck Sharp & Dohme Pharmaceuticals Inc. Istanbul, Turkey) via the orogastric probe within 2 cc running water. Using the same method 2 cc running water was applied to the control group. 48 hours later, the rats were prepared for surgery under clean but not sterile conditions with an injection of 50 mg/kg dose of IM ketamine hydrochloride anesthesia (Ketalar, Pfizer Pharmaceuticals Inc. Istanbul, Turkey). After hair removal, the abdomens were cleaned with povidone-iodine solution and a 3 cm midline laparotomy were made. Following the laparotomy, at both sides of the abdominal wall, 3 pieces of 0.5 cm peritoneal tissues were stitched with the 4/0 silk from its base one by one by using hemostat and an ischemic peritoneal tissue was created. The abdominal cavity was closed by 3/0 prolen at the anatomic plane. In the following 15 days simvastatin was applied to the study group (Group I) within 2 cc running water via orogastric probe and 2 cc running water was applied to the control group (Group II) via orogastric probe. The rats were fed ad libitum.

15 days after the surgery all of the rats were sacrificed with the high dose ether inhalation. The abdominal cavity was opened via an inverted U-shaped incision. The abdomen was washed with 1 cc physiological saline solution and re-aspirated with a syringe. Status of intra-abdominal adhesions were evaluated macroscopically by an investigator blinded to study and adhesions were scored. Peritoneal samples were taken from areas with adhesion for pathological analyses. These samples were fixed in a 10% buffered formaldehyde solution, and after routine tissue processes parafin blocks were prepared. The serial sections were stained with hematoxylin eosin and microscopic evaluations were carried out.

The adhesion scores were determined in terms of the degree of adhesion of intraabdominal organs and omentum to ischemic buttons: 0 - no adhesion, 1 - easily detachable adhesion, 2 - adhesion detachable by traction, 3 - adhesion detachable by sharp dissection. Percentages of adhesion was calculated by comparing the the proportion of the ischemic buttons with adhesions to all buttons. Score for general adhesion was found by multiplying the percentages of adhesion to adhesion scores (percentage of adhesion x adhesion score = overall adhesion value).

Microscopic evaluation of adhesion was assessed under 5 main topics which are the signs of inflammatory response; inflammation, activation, fibroblast activity, vascularity, presence of giant cell. Activation was scored as follows: 0 - no activation, 1 - while activation was accepted as present the score for other parameters was evaluated between 0 to 3 according to the increased severity. After evaluating all topics separately, the average of all scores has been assessed in both groups.

Statistical analysis was performed using the SPSS (Statistical Package for Social Science) for Windows, Release 11.5. Taking into account the number of subjects and distribution, the results were evaluated using a Chi-square test and Mann-Whitney U test. $p<0.05$ was considered statistically significant.

Results

As a result of the macroscopic evaluation of postoperative intra-abdominal adhesions, the percentage of adhesion in simvastatin applied group was found to be 0.8 ± 0.17. This value was calculated as 0.6 ± 0.2 in the control group. Regarding the severity of adhesion, while in the simvastatin applied group the value was found to be 9.1 ± 4, in the control group it was 6.8 ± 3. The general adhesion score was found to be 7.7 ± 4.2 in simvastatin applied group and 5.1 ± 3.7 in control group. Comparing the two groups according to adhesion scores no significant difference was observed between the study and control group. The summary of results were shown in **Table 1**.

Table 2. Inflammation severity in Group I and Group II

Inflammation severity	Grade 1	Grade 2	Grade 3
Simvastatin	6 (50%, 0)	4 (33%, 3)	2 (16%, 7)
Control	5 (62%, 5)	2 (25%, 0)	1 (12%, 5)

Table 3. The values of fibroblastic activity in Group I and Group II

Fibroblastic activity	Grade 1	Grade 2	Grade 3
Simvastatin	25	42	33
Control	62	38	0

Table 4. Vascularity changes and grades in Group I and Group II

Vascularity	Grade 1	Grade 2	Grade 3
Simvastatin	42	50	8
Control	38	50	12

Table 5. Giant cell formation scores in microscopic examination

Giant cell (%)	Grade 0	Grade 1	Grade 2	Grade 3
Simvastatin	17	58	8	17
Control	12	88	0	0

In histopathological evaluation, group I and group II were statistically compared in terms of the degree of inflammation, the presence of activation, degrees of fibroblast activity, the extent of increase in vascularity, the degree of the amount of giant cells. The inflammation score in simvastatin group was determined as follows; grade 1 - 50%, grade 2 - 33.3%, grade 3 - 16.7%, in control group it was determined as follows; grade 1 - 62.5%, grade 2 - 25%, grade 3 - 12.5. Statistically no difference was observed between two groups ($p>0.05$) (**Table 2**).

While investigating the presence of activation in the samples of both groups, in 58.3% of the simvastatin group activation was determined, and in 41.7% of the group no activation determined. Regarding the control group in 37.5% of the group activation was determined while in 62.5% of the group no activation was determined. Statistically no significant difference was observed between two groups ($p>0.05$).

In terms of fibroblast activity values, following percentages were observed in simvastatin group; grade 1 - 25%, grade 2 - 41.7%, grade 3 - 33.3%. These values were determined in the control group as follows; grade 1 - 62.5%, grade 2 - 37.5%, grade 3 - 0%. In terms of fibroblast activity statistically no significant difference was observed between two groups (**Table 3**).

In evaluation of the scores of increase in vascularity, the following results were obtained in simvastatin group: grade 1 - 41.7%, grade 2 - 50%, grade 3 - 8.3%, and in control group: grade 1 - 37.5%, grade 2 - 50%, grade 3 - 12.5%. Statistically no significant difference was observed between two groups **Table 4**). The giant cell score was determined in simvastatin group as follows; grade 0 - 16.7%, grade 1 - 58.3%, grade 2 - 8.3%, grade 3 - 16.7%, and in control group it was determined as follows; grade 0 - 12.5%, grade 1 - 87.5%, grade 2 - 0%, grade 3 - 0%. Statistically no significant difference was observed between two groups (**Table 5**).

Discussion

Abdominal adhesion formation after abdominopelvic surgery is a condition that causes significant clinical and financial problems. In long term follow-up studies it has been observed that postoperative abdominal adhesions cause small intestine obstructions, chronic pelvic pain, infertility and difficulties in the following operations. Although new surgical techniques, anti-inflammatory drugs, fibrinolytic agents, antibiotics and synthetic protective barriers have been used to prevent postoperative adhesion formation, none of them were proved to be fully and consistently effective. A better grasp of the mechanisms of peritoneal healing and molecular mechanisms involved in adhesion formation in recent years expedites the search for a more simple and effective method [4].

The adhesion formation starts with a trauma to the surface of the peritoneum. The presence of ischemia and inflammation accompanying the local damage to the peritoneum inhibits the fibrinolytic system and leads to the formation of inflammatory exudate and fibrin bands. With the invasion of inflammatory cells and fibroblasts these bands became persistent fibrin bands. The plasminogen is converted to plasmin by the action of plasminogen activators

(t-PA and u-PA). The plasmin is responsable for the degredation of fibrin bands. t-PA is synthesized in the abdomen by the mesothelial cells and inactivated by plasminogen activator inhibitor 1 (PAI-1) [13-15].

Simvastatin is an inhibitor of HMG-CoA reductase, which is a rate-limiting enzyme in cholesterol synthesis. In clinical studies Simvastatin is used as an effective lipid-lowering drug. Recent studies showed that besides the serum cholesterol-lowering effect of simvastatin, it has antiinflammatory, antioxidant, fibrinolytic effects independently [16, 17]. These effects are known to be important to prevent abdominal adhesions. The fibrinolytic effect of simvastatin on endothelial cells [18, 19], human vascular smooth muscle cells [20], rabbit renal mesangial cells [21] has been shown. In their in vitro human mesothelial cell culture experiments, Haslinger et al showed that simvastatin stimulates fibrinolytic activity by significantly increasing the t-PA level and reducing the PAI-1 level [18]. One year later Haslinger et al. published another study showing that simvastatin is a stimulant of fibrinolytic system in the mesothelial cells stimulated by TNF-α [22]. Since this model is considered to mimic intraabdominal inflammation in vitro, it is thought that simvastatin might stimulate fibrinolytic activity in inflammatory cases as it does in normal peritoneal cells. The positive effects of statins on treatments in other models of sepsis were shown by Mark W. Merx et al [23]. These studies conducted on tissue cultures provided a basis for our study to test simvastatin on living organism in order to prevent adhesion formation. For years simvastatin is used in clinical practice due to its lipid-lowering effect. Simvastatin tablet for oral administration is convenient and inexpensive. Therefore, we investigated the effect of orally administered simvastatin on the postoperative adhesion formation, making use not of its lipid lowering effect but its fibrinolitic effects shown in studies in vitro. In our study, simvastatin 40 mg/kg orally was started to be applied 48 hours before surgery. After the surgery of adhesion formation with the ischemic button model, we continued to administer simvastatin for 15 days.

Since the oral form of this drug is in common use, and its clinical pharmacokinetic qualities is defined in detail both in rats and human beings we prefered to administer this drug orally. The efficiency and reliability of the oral intake of simvastatin as a lipid-lowering drug has been proved. As a fibrinolytic agent the same drug intake method is thought to be a more appropriate method for cases with possible intraabdominal adhesion diagnosis. In intraperitoneal application, the drug instilled into the peritoneum composed of the adjuvants and granules might be perceived as a foreign body inside the abdomen and might stimulate the adhesion. The absorption of drugs that will be instilled into the peritoneum in order to prevent intraabdominal adhesion will be fast because the circulation inside the peritoneum is very fast, therefore, the drug will have no long term effect on peritoneal mesothelial cells, which it is supposed to affect. In addition, since only single dose intraperitoneal drug administration is possible, the durability of the drug efficacy wouldn't be possible. Therefore, in order to increase the efficacy of the drug on peritoneal mesothelial cells, in our study the drug is administered to the rats 48 hours before the laparotomy so that the drug will be in highest concentration in the tissue during the period of adhesion formation. The reason for high dose drug application such as 40 mg/kg was to generate the fibrinolytic effect of simvastatin, if present, in maximum concentration.

In this study it has been shown that 40 mg/kg dose oral administration of simvastatin to rats has no effect on the intraabdominal adhesion which were induced via ischemic button model. No significant statistical difference was determined between the simvastatin applied group and the control group neither in terms of the grading and percentage of the adhesion nor total adhesion scorings.

In discussion section it has been mentioned that the study of Aarons et al. showed that intraperitoneal administrations of statins prevents intraabdominal adhesions [24]. 24 hours after the single dose 30 mg/kg intraperitoneal lovastatin and atorvastation (member of statin class pharmaceuticals) application, the researchers measured t-PA and mRNA levels in the peritoneal tissue and the t-PA activity within the peritoneal fluid. 7 days later rats were sacrified and the intraabdominal adhesion was evaluated macroscopically. The results showed that after the intraperitoneal administration of atorvastatin and lovastatin, t-PA and mRNA levels and t-PA activity in peritoneum increased sig-

nificantly and macroscopic evaluations showed that adhesion was reduced in those rats [24]. There are several reasons for the differences between the results of the study of Aaron et al and our study. First of all differences in route of administration of the drug can affect the results. Orally administered drugs are first metabolized in the gastrointestinal system and liver, and then they enter to the systemic circulation. In intaperitoneal administrations the drug produces its effect locally with diffusion. Another factor is that Aarons et al used atorvastatin and lovasttatin in their experimental models [24]. Although these drugs together with simvastatin constitute the statin group there are differences in their biochemical structures. Even these differences are not very important in terms of their lipid-lowering effect, their fibrinolytic effect might be different since their pharmacokinetic properties are different. Therefore fibrinolytic effect indicated with atorvastatin and lovastatin may not be indicated with simvastatin. Orally administered drugs are first metabolized in the gastrointestinal system and liver, and then they enter to the systemic circulation. In intaperitoneal administrations the drug produces its effect locally with diffusion. Another factor is that Aarons et al used atorvastatin and lovasttatin in their experimental models [24]. The concentration of drug applied directly to the peritoneal tissue is higher compared to the dosage applied orally which reaches to the peritoneum after being metabolized in the body. Since the induction of t-PA might occur depending on the dosage, intraperitoneal usage might lead to more t-PA induction. Finally it has been observed that intraperitoneal and oral administrations of drugs have different effects on adhesion. In a study flavonoids were used to prevent postoperative adhesion formation however while the drug instilled to the peritoneum reduced the formation of adhesion orally administered drug did not reduced the adhesion formation [25].

In literature the antiinflammatory effect of simvastatin has been shown in different study models. However in some of the recent studies no antiinflammatory effect of simvastatin has been observed indeed it has been observed that simvastatin increases inflammation. It has been shown that simvastatin increases IL-12 and TNF alpha in lipopolisaccharide-stimulated rat macrophages in promoter level and it was discussed that this situation can provide a basis for bacterial infections [26]. In addition, in a clinical study it has been shown that simvastatin has no antiinflammatory effect on asthma patients treated with this drug [27]. In our study no antiinflammatory effect of orally administered simvastatin on peritoneum has been observed. No significant difference was observed between two groups in terms of fibroblast activity, the presence of giant cell, increase in vascularity, the degree of inflammation and the activation in adhesion tissues evaluated microscopically for inflammation.

Simvastatin is used in clinical practise as a lipid lowering drug and the experimental studies proved its antiinflammatory and fibrinolytic effect. In our study simvastatin was tested in an adhesion model on rats with 40 mg/kg dose administered orally. In this experimental study it was showed that orally administered simvastatin has no effect on preventing formation of postoperative adhesions. At the same time, it has been observed that this dose of simvastatin has also no anti-inflammatory effect on preventing postoperative adhesion formation.

Disclosure of conflict of interest

None.

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References

[1] Stricker B, Blanco J, Fox HE. The gynecologic contribution to intestinal obstruction in females. J Am Coll Surg 1994; 178: 617-20.
[2] Ellis H. The clinical significance of adhesions: focus on intestinal obstruction. Eur J Surg Suppl 1997; 5-9.
[3] Punch MR, Roth RS. Adhesions and chronic pain: an overview of pain and a discussion of adhesions and pelvic pain. Prog Clin Biol Res 1993; 381: 101-20.
[4] Risberg B. Adhesions: preventive strategies. Eur J Surg Suppl 1997; 32-9.
[5] Hellebrekers BW, Trimbos-Kemper TC, Trimbos JB, Emeis JJ, Kooistra T. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. Fertil Steril 2000; 74: 203-12.
[6] Luciano AA, Maier DB, Koch EI, Nulsen JC, Whitman GF. A comparative study of postoper-

ative adhesions following laser surgery by laparoscopy versus laparotomy in the rabbit model. Obstet Gynecol 1989; 74: 220-4.
[7] Bulbuloglu E, Ezberci F, Gul M, Kurutas EB, Bozkurt S, Kale IT, Ciragil P. Effects of the intraperitoneal lornoxicam on the formation of intraperitoneal adhesions in rat peritonitis model. ANZ J Surg 2005; 75: 1115-9.
[8] Celebioglu B, Eslambouli NR, Olcay E, Atakan S. The effect of tenoxicam on intraperitoneal adhesions and prostaglandin E2 levels in mice. Anesth Analg 1999; 88: 939-42.
[9] Oncel M, Kurt N, Remzi FH, Sensu SS, Vural S, Gezen CF, Cincin TG, Olcay E. The effectiveness of systemic antibiotics in preventing postoperative, intraabdominal adhesions in an animal model. J Surg Res 2001; 101: 52-5.
[10] Beck DE. The role of Seprafilm bioresorbable membrane in adhesion prevention. Eur J Surg Suppl 1997; 49-55.
[11] Pagidas K, Tulandi T. Effects of Ringer's lactate, Interceed(TC7) and Gore-Tex Surgical Membrane on postsurgical adhesion formation. Fertil Steril 1992; 57: 199-201.
[12] Kresch AJ, Seifer DB, Sachs LB, Barrese I. Laparoscopy in 100 women with chronic pelvic pain. Obstet Gynecol 1984; 64: 672-4.
[13] Holmdahl L, Eriksson E, al-Jabreen M, Risberg B. Fibrinolysis in human peritoneum during operation. Surgery 1996; 119: 701-5.
[14] Hellebrekers BW, Emeis JJ, Kooistra T, Trimbos JB, Moore NR, Zwinderman KH, Trimbos-Kemper TC. A role for the fibrinolytic system in postsurgical adhesion formation. Fertil Steril 2005; 83: 122-9.
[15] Bouckaert PX, Land JA, Brommer EJ, Emeis JJ, Evers JL. The impact of peritoneal trauma on intra-abdominal fibrinolytic activity, adhesion formation and early embryonic development in a rabbit longitudinal model. Hum Reprod 1990; 5: 237-41.
[16] Deakin S, Leviev I, Guernier S, James RW. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. Arterioscler Thromb Vasc Biol 2003; 23: 2083-9.
[17] Sukhova GK, Williams JK, Libby P. Statins reduce inflammation in atheroma of nonhuman primates independent of effects on serum cholesterol. Arterioscler Thromb Vasc Biol 2002; 22: 1452-8.
[18] Haslinger B, Goedde MF, Toet KH, Kooistra T. Simvastatin increases fibrinolytic activity in human peritoneal mesothelial cells independent of cholesterol lowering. Kidney Int 2002; 62: 1611-9.
[19] Haslinger B, Kleemann R, Toet KH, Kooistra T. Simvastatin suppresses tissue factor expression and increases fibrinolytic activity in tumor necrosis factor-alpha-activated human peritoneal mesothelial cells. Kidney Int 2003; 63: 2065-74.
[20] Wiesbauer F, Kaun C, Zorn G, Maurer G, Huber K, Wojta J. HMG CoA reductase inhibitors affect the fibrinolytic system of human vascular cells in vitro: a comparative study using different statins. Br J Pharmacol 2002; 135: 284-92.
[21] Wei J, Ma C, Wang X. Simvastatin inhibits tissue factor and plasminogen activator inhibitor-1 expression of glomerular mesangial cells in hypercholesterolemic rabbits. Biomed Res 2006; 27: 149-55.
[22] Bea F, Blessing E, Shelley MI, Shultz JM, Rosenfeld ME. Simvastatin inhibits expression of tissue factor in advanced atherosclerotic lesions of apolipoprotein E deficient mice independently of lipid lowering: potential role of simvastatin-mediated inhibition of Egr-1 expression and activation. Atherosclerosis 2003; 167: 187-94.
[23] Merx MW, Liehn EA, Graf J, van de Sandt A, Schaltenbrand M, Schrader J, Hanrath P, Weber C. Statin Treatment After Onset of Sepsis in a Murine Model Improves Survival. Circulation 2005; 112: 117-24.
[24] Aarons CB, Cohen PA, Gower A, Reed KL, Leeman SE, Stucchi AF, Becker JM. Statins (HMG-CoA reductase inhibitors) decrease postoperative adhesions by increasing peritoneal fibrinolytic activity. Ann Surg 2007; 245: 176-84.
[25] Kafkaslı A, Alataş E. The effect of intraperitoneal flavonoid (Daflon 500 mgr) treatment for prevention of adhesion formation. Journal of Turgut Özal Medical Center 1999; 6: 1-5.
[26] Matsumoto M, Einhaus D, Gold ES, Aderem A. Simvastatin augments lipopolysaccharide-induced proinflammatory responses in macrophages by differential regulation of the c-Fos and c-Jun transcription factors. J Immunol 2004; 172: 7377-84.
[27] Menzies D, Nair A, Meldrum KT, Fleming D, Barnes M, Lipworth BJ. Simvastatin does not exhibit therapeutic anti-inflammatory effects in asthma. J Allergy Clin Immunol 2007; 119: 328-35.