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
Effect of simvastatin on adhesion formation

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 prolene 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 intra-abdominal 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 1. Comparison of adhesion characteristics between Group I and Group II

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin (Group I)</th>
<th>Control (Group II)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion severity</td>
<td>0.8</td>
<td>0.6</td>
<td>0.200</td>
</tr>
<tr>
<td>Adhesion rate</td>
<td>9.1</td>
<td>6.8</td>
<td>0.136</td>
</tr>
<tr>
<td>General adhesion score</td>
<td>7.7</td>
<td>5.1</td>
<td>0.141</td>
</tr>
</tbody>
</table>

Table 1: Comparison of adhesion characteristics between Group I and Group II
Effect of simvastatin on adhesion formation

In histopathological evaluation, group I and group II were statistically compared in terms of the degree of inflammation, the presence of activation, 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 - 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

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### Table 2. Inflammation severity in Group I and Group II

<table>
<thead>
<tr>
<th>Inflammation severity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>6 (50%, 0)</td>
<td>4 (33%, 3)</td>
<td>2 (16%, 7)</td>
</tr>
<tr>
<td>Control</td>
<td>5 (62%, 5)</td>
<td>2 (25%, 0)</td>
<td>1 (12%, 5)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fibroblastic activity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>25</td>
<td>42</td>
<td>33</td>
</tr>
<tr>
<td>Control</td>
<td>62</td>
<td>38</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Vascularity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>42</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>50</td>
<td>12</td>
</tr>
</tbody>
</table>

### Table 5. Giant cell formation scores in microscopic examination

<table>
<thead>
<tr>
<th>Giant cell (%)</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>17</td>
<td>58</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>88</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Effect of simvastatin on adhesion formation

(t-PA and u-PA). The plasmin is responsible for the degradation 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 anti-inflammatory, 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 fibrinolytic 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 preferred to administer this drug oral-ly. 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 sacrificed 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-
Effect of simvastatin on adhesion formation

Significantly 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 lovastatin 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 lovastatin 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 reduce 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


