

Comparison of the Efficacy of Oral Simvastatin and Topical Simvastatin Solution in Decreasing Post-Laparotomy Adhesions in Rats

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Abstract

Background: Intra-abdominal adhesions and their complications occur frequently after laparotomy. The aim of this study was to compare oral versus intraperitoneal administration of simvastatin in decreasing post-laparotomy adhesions in rat.

Methods: Thirty male Wistar albino rats were divided into three groups of ten. All the rats underwent laparotomy and induction of adhesions using the method of Meso-Stitch approximation of injured cecum and abdominal wall. One group received oral simvastatin (40 mg/kg) daily during two weeks before the laparotomy. In the two other groups, 2 ml of solution of simvastatin (40 mg/kg) or distilled water (as placebo) was spilled into abdomen before closing abdominal wall, respectively. After 14 days, all the rats were put under laparotomy again to be compared. Rates and grades of adhesions were assessed using Hoffman et al. and Lauder et al. Scale and histopathological reports.

Results: In placebo group, the grade II and III adhesion was seen in 2 and 8 rats, respectively. In local simvastatin group, there was no adhesion in 5 rats, and grade I and II adhesion was seen in 3 and 2 rats, respectively. In oral simvastatin group, 6 rats were without adhesion, and 3 cases with grade I and 1 case with grade II adhesion. The frequency and grade of adhesion were statistically different in simvastatin groups compared to the placebo group ($P < 0.001$), but not with each other.

Conclusions: Oral simvastatin for two weeks before the laparotomy can reduce post-laparotomy adhesion bands as well as local administration of simvastatin solution.

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Introduction

Annually, over 300000 patients undergo surgery for the treatment of intestinal obstruction due to adhesions in the United States (1) and 20-years analysis (1988 to 2007) did not show any reduction in this amount (2).

Although all the patients with adhesion do not show clinical signs, but early or late intestinal obstruction (49-79 percent), chronic abdominal pain (20-70 percent) and infertility (15-20 percent) are considered as the major surgical problems (1).

The studies using serial laparoscopies showed that at the 8th day after the surgery, 55% of the adhesions are appeared and 100% of them were visible between the 4th to 8th weeks (3,4). Although laparoscopy and

microsurgery techniques have reduced the formation of adhesions, but the benefits of adjuvant use of different substances, despite their widespread use, is still questionable (5).

Many drugs, such as anti-inflammatory or fibrinolytic agents and physical barriers are recommended; but there is still no ideal substance to prevent adhesions. Hyaluronic acid, Intergel (hyaluronic acid binding ferric ion), Sepracoat (combination of hyaluronic acid and carboxymethyl cellulose), Hydrogel (spray gel), Viscogels and statins are of the substances used to reduce the adhesion (6-8). The only drug that is partly been proven to reduce the adhesion is the hyaluronate that is not available to everyone and interferes in the restoration of

anastomoses (1). Therefore, efforts are ongoing to examine other factors in reducing adhesions. Among these factors, are the statins that have anti-inflammatory and fibrinolytic effects (9).

According to the our previous study and the positive effect of local simvastatin at a dose of 30 mg per kg of body weight in preventing the formation of adhesion bands in rats (6), and based on similar studies and the significant effect of oral statins prior to laparotomy in this animal model (8), this study aimed to investigate the anti-adhesion effect of oral and intraperitoneal simvastatin, compared to placebo (distilled water) in vivo conditions.

Materials and Methods

In this experimental study, 30 adult male Wistar albino rats weighing 250 to 300 g with the age of 4-6 months and the same race were selected and divided into three groups of ten. In the first group, during two weeks before the study, oral simvastatin HEXAL (Germany) with the dose of 40 mg/kg dissolved in water was administrated daily.

This study was done under the regarding the Guide for the Care and Use of Laboratory Animal provided by Shahid Beheshti Laboratory Animals Center in accordance with global standards for laboratory biosafety guidelines (10). The rats were housed in the animal nest of the Department of Anatomy in School of Medicine. The study was approved by the institutional review board and the Ethics Committee of Shahid Beheshti University of Medical Sciences, Iran.

The rats were intramuscularly anesthetized with ketamine 60 mg/kg ROTEXMEDICA (Germany) and then, underwent midline laparotomy incision with the length of 4 cm. In all three groups, powder-free gloves were used during the surgery. To create adhesion, according to Daniel study, the method of Meso-Stitch approximation of injured cecum and abdominal wall was used in this way: scratching with a blade on the cecum and creating one-square-centimeter defects on the abdominal wall and creating sutures for abdominal wall and the skin with 3-0 nylon (11). All the rats underwent the surgery in one day.

At the end of the surgery, in the second and groups, before closing the abdomen, 2 cc of simvastatin solution (40 mg/kg) and 2 cc distilled water as placebo was spilled into abdomen, respectively, and then the abdomen was closed. For the first group, that had received drug for two weeks, nothing was administrated in this step.

After two weeks, the rats were reevaluated in terms of adhesions. They were anesthetized using ketamine and then euthanized using chloroform gas; re-laparotomy was performed and adhesions in the three groups were compared via observation during the surgery based on the quantitative questionnaire of

Hoffman et al. use by Poehnert et al. (11), observation during surgery based on the qualitative questionnaire of Lauder et al. (12), and histological reports (biopsy).

The degree of clinical adhesion was scaled as: 0: No adhesion, 1: One adhesion band, no vessel, easily separated, 2: Two thin adhesion bands, no vessel, easily separated, 3: Three thin adhesion bands, no vessel, easily separated, and 4: More than three thin adhesion bands, easily separated with no vessel or diffuse adhesion bands with vessels (13).

The degree of pathological adhesion was scaled as: 0: No adhesion, 1: Fat (Figure 1), 2: Fat and fibrosis, and 3: Fibrosis (Figure 2).



Figure 1. Fourth-degree intra-abdominal adhesion in a rat receiving normal saline (control group). Note the intraperitoneal adhesion between the colon and the mesothelium of small intestine. More than three thin adhesion bands, easily separated with no vessel or diffuse adhesion bands with vessels

The obtained data were analyzed using Kruskal-Wallis and Mann-Whitney U evaluated using SPSS software (version 20, IBM Corporation, Armonk, NY) at the significant level of $P < 0.050$.



Figure 2. First-degree intra-abdominal adhesion in a rat receiving normal saline (local simvastatin group). Note the intraperitoneal loose adhesion between the colon and the mesothelium of small intestine. One adhesion band, no vessel, easily separated

Results

Totally, 30 male Wistar albino rats weighing 250-300 g with the age of 5-6 months underwent were included in the study. The duration of each surgery was 20 minutes. No mortality was recorded. The volume of intraoperative bleeding was comparable between groups.

Table 1. Comparison of the degree of clinical adhesion between the study groups

The degree of clinical adhesion	Local simvastatin	Oral simvastatin	Placebo
	[n (%)]	[n (%)]	[n (%)]
0: No adhesion	5 (50)	6 (60)	0 (0)
1: One adhesion band, no vessel, easily separated	3 (30)	1 (10)	0 (0)
2: Two thin adhesion bands, no vessel, easily separated	2 (20)	3 (30)	0 (0)
3: Three thin adhesion bands, no vessel, easily separated	0 (0)	0 (0)	2 (20)
4: More than three thin adhesion bands, easily separated with no vessel or diffuse adhesion bands with vessels	0 (0)	0 (0)	8 (80)

P < 0.001 between the both simvastatin groups and placebo group, P = 0.910 between the simvastatin groups

Clinical finding showed that in placebo group, 8 cases had grade 4 and 2 rats had grade 3 adhesions; while in intra-abdominal and oral simvastatin groups, 5 and 6 cases did not have adhesion, respectively. Based on the clinical findings, the formation of severe adhesions in the oral and topical simvastatin groups was significantly less than the placebo group (P < 0.001); but no significant difference was observed between the two simvastatin groups (P = 0.910) (Table 1).

Based on the pathological findings, the formation of fibrosis in the oral and topical simvastatin groups was significantly less than the placebo group (P < 0.001); there were no significant difference between the two simvastatin groups (P = 0.907) (Table 2).

Discussion

The findings of this study showed that oral and local administration of simvastatin can reduce the formation of intra-abdominal adhesions following laparotomy. Arons et al., in an experimental study on rats, examined the effect of statins in the prevention of adhesion and its effect on wound healing. Oral and topical atorvastatin (30 mg/kg) were administrated into two groups of 15 rats. After 7 days, repeated laparotomy showed that only in the topical type, adhesion formation reduced (3). This is not consistent with the findings of this investigation in oral form. In this study, simvastatin with a dose of 40 mg/kg was used and in both oral and topical groups, decreased clinical and pathological adhesion after 14 days.

Hoşcan et al. study was conducted on 40 rats with fluvastatin. In the group of intra-abdominal fluvastatin with dosage of 10 mg/kg, all the rats died after one day and this group was excluded from the study. But in the oral group with the same dosage, reduction in adhesion was observed after two weeks (9). In our study, simvastatin was used and none of the rats died. Perhaps, because of the influence of different groups of different

classes of statins, all the rats which received topical fluvastatin, died Actachir et al. study. This is the reason that simvastatin was used in the present study.

Kucuk et al. by administering 0.57 mg/kg intraperitoneal simvastatin and measurement of tissue-type plasminogen activator (t-PA) in the abdomen in 30 rats, after a week, found that reducing adhesions in the abdominal cavity is associated with increased levels of t-PA (13). In the present study, the adhesion criteria was the clinical and histological observation and laboratory criteria were not used.

Jahovic et al. showed that the two drugs of simvastatin and fluvastatin were not similar in decreasing inflammatory parameters in colitis in two groups of 10 rats. Fluvastatin decreased the colon tissue collagen content and the level of tumor necrosis factor (TNF) more (14). In the case of simvastatin, the capacity of hydroxyl radicalism is more, while fluvastatin had higher antioxidants for taking the anti-peroxyl radicals (15). Fluvastatin, decreased the monocyte CD11b expression and endothelial adhesion, too (16).

In Hoşcan et al. study, the effect of fluvastatin on wound healing was measured by wound hydroxyproline content which did not differ with the control sample and the effect of simvastatin on the restoration of tissues was not studied (9). This is one of the issues that must be evaluated for assessment of the effects of drug on the anastomosis to determine whether topical or oral simvastatin impairs healing or not.

Yildiz et al. showed that oral simvastatin had no effect in reducing adhesions (8); their findings are not consistent with this study. In the Yildiz et al. study, simvastatin was given just 48 hours before the laparotomy, whereas in the present study, the medication was given 14 days prior to surgery (8). Another difference is that they performed another laparotomy 7 days later, while this was done after 14 days in the present study.

Table 2. Comparison of the degree of pathological adhesion between the study groups

The degree of pathological adhesion	Local simvastatin [n (%)]	Oral simvastatin [n (%)]	Placebo [n (%)]
0: No adhesion	5 (50)	6 (60)	0 (0)
1: Fat	3 (30)	1 (10)	0 (0)
2: Fat and fibrosis	2 (20)	3 (30)	2(20)
3: Fibrosis	0 (0)	0 (0)	8 (80)

P < 0.001 between the both simvastatin groups and placebo group, P = 0.907 between the simvastatin groups

The adhesion criterion in that study was the activity of t-PA, which did not increase in oral form. Although the dose of simvastatin was similar to the present study, but perhaps with 48 hours of oral medication, the appropriate level of the drug was not created in mesothelial cells.

In a pilot study on human model, analyzing the data of 46 patients on oral simvastatin for 3-7 days before the surgery did not show any significant difference on the level of t-PA and plasminogen activator inhibitor-1 (PAI-1) compared to control group at the first day after the surgery (17). The shortness of sample size as well as the low duration of drug administration, were the limitations of that pilot study.

In another human study, oral administration of 40 mg of simvastatin from 3-7 days before the surgery to 4 days after it, tended to significant decrease of inflammatory factors (18).

Haslinger et al. in in-vitro study on mesothelial cells showed that simvastatin stimulated fibrinolytic activity with increasing levels of t-PA and decreasing PAI-1 (19). In another study, Bea et al. showed that simvastatin was the stimulant of fibrinolytic system in mesothelial cells by reducing TNF-a (20). We showed the effect of local simvastatin (30 mg/kg body weight) in reducing adhesion, with a significant difference compared to the control group in a sample of 32 rats with clinical and pathological criteria (6).

Conclusion

The present study showed that oral simvastatin similar to topical simvastatin was significantly effective in reducing adhesion compared to the control group in rats. More studies are also needed on human subjects to examine the effects on healing of anastomosis.

Conflict of Interests

Authors have no conflict of interests.

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