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Original Article

Comparison of the Effects of Atorvastatin, Hyaluronic Acid and Oxidized Cellulose (Interceed) on Reducing Intestinal Adhesions Postoperative after Open Abdominal Surgery in Animal Models

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Abstract

Abdominal adhesions are considered as an important problem in abdominal surgery. In this study we compared the effectiveness of atorvastatin, hyaluronic acid and interceed (oxidized cellulose) in prevention of postoperative intestinal adhesions. A total of 40 Wistar-albino rats were randomly divided into 5 groups: Group 1 (Sham); Group 2 (Control or Adhesion induction); Group 3 (Adhesion induction + hyaluronic acid); Group 4 (Adhesion induction + oxidized cellulose); and Group 5 (Adhesion induction + atorvastatin). A same experimental method of cecal abrasion and ligature was used to produce adhesions in all rats. Fourteen days after adhesions formation, animals were killed and adhesions were scored according to macromorphological characteristics and adhesion-carrying tissues under standard histologic examination. Inflammation, vascularization and fibrosis in granulation sites were graded in all samples. Adhesion scores in all experimental groups were slightly lower than control group. Group 3 had the lowest mean adhesions score (1.18 ± 1.24) based on surgeon observation. The mean of adhesion score in Group 3 (1.18 ± 1.24) was significantly lower than that in Group 4 (3.43 ± 0.53) ($p=0.004$). Group 5 showed the lowest mean adhesions score on pathological examination (5.62 ± 3.73). The mean of adhesion total score in Group 3 was slightly lower than that in other groups; however, this difference was not significant. Hyaluronic acid, oxidized cellulose, and atorvastatin are effective in treatment and prevention of intestinal adhesions during open abdominal surgery. Although hyaluronic acid was slightly more effective, further studies are needed to evaluate the effect of combination therapy with these agents.

Keywords: Atorvastatin, hyaluronic acid, oxidized cellulose, intestinal adhesions

1. Introduction

Abdominal adhesions are abnormal connections between tissues and organs which can be caused by multiple factors such as inflammation, damages to the peritoneal area, surgical trauma, and endometriosis [1]. Abdominal adhesions are considered as the most common cause of intestinal obstructions [2]. The incidence of this abnormality usually ranges from 67% to 93% after abdominal

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surgery [3]. They bring huge financial burden to the patient's families and societies, and also cause various problems such as chronic pain, chronic abdominal pain, female infertility and bowel obstruction in affected patients [4]. More importantly, the frequency of this complication may increase along with the number of previous abdominal surgeries.

Over the past few decades, a large number of studies have been focused on seeking effective approaches on post-surgery adhesion. Several drugs such as anti-inflammatory and antithrombotic are commonly used to avoid adhesions. Nevertheless, because of their fast clearance in abdominal cavity and several side effects, a limited number of these drugs can be applied in clinical practices [5]. Mechanical barriers are the other strategies that separate damaged tissues from injured areas during the healing process. These barriers are usually divided into two groups, including liquid (e.g. dextran, hyaluronic acid and crystalloids) and solid barriers (e.g. Interceed®, Seprafilm® and SprayGel®) [6, 7]. The persistent time for liquid barriers is too short for the maintenance of their efficacy [8]. It is difficult to handle and fix solid barriers to the damaged tissues because some of them can aggressively adhere to moisture such as the surgeon's gloves [9]. Therefore, it is still a challenge to find a better kind of anti-adhesion drug or material which is safe, effective, easy to use, and has an appropriate gelation and retention time [3].

At present, various anti-adhesion agents, such as hyaluronic acid, oxidized cellulose, icodextrin and atorvastatin are available for clinical applications. Hyaluronic acid (HA) is a natural glucose amino glycan that constitutes a major part of the extracellular matrix such as skin, connective tissues, cartilage, and synovial liquids [10]. It has the ability to coat and lubricate serosal surfaces. It also potentially prevents serosal trauma during surgery, as well as postoperative formation of adhesions in the abdomen [3]. Oxidized cellulose is a solid barrier that can inhibit adhesions formation after abdominal surgery [11]. Statins are a family of drugs that have cardiovascular protective effects by inhibiting the hydroxyl-methyl-glutaryl-coenzyme-A reductase (HMG-COA) [12]. They have also anti-inflammatory and antioxidative properties which increase microvascular activity [13]. Experimental studies showed that statins, such as atorvastatin, have anti-adhesion activity by increasing the activity of peritoneal fibrinolytic [12].

In this study, we compared the effects of atorvastatin, hyaluronic acid and oxidized cellulose in the prevention or treatment of peritoneal adhesions in Wistar rats.

2. Materials and Methods

2.1. Animals

This experimental study was conducted in Baqiyatallah University of Medical Science after approval by the local ethical committee. Forty Wistar-Albino male rats with weights ranging between 200 and 250 g (7 weeks of age) were randomly divided into 5 groups: Group 1: Sham; Group 2: Control (Adhesion induction); Group 3: Adhesion induction + hyaluronic acid (Sanct Bernhard, Germany; P-10834); Group 4: adhesion induction + oxidized cellulose (MEDITAMP® EXTRA); Group 5: Adhesion induction + atorvastatin (Atorvastatin-ARYA 20mg Tab).

2.2. Surgical Procedure

All animals were anesthetized with intraperitoneal administration of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg). In group 1, the abdominal incision was closed without any adhesion intervention. Following laparotomy via a 3 cm midline incision, adhesion formation model was used according to the modified protocol of Hemadeh *et al* [14]. A gauze sponge was rubbed on the serosa of the cecum until the serosal shine was lost and punctuate hemorrhagic spots oozing blood were formed. The cecum was then returned to the abdomen (Figure 1). In Group 2, the abdomen was closed after adhesion formation method without any further treatment. In

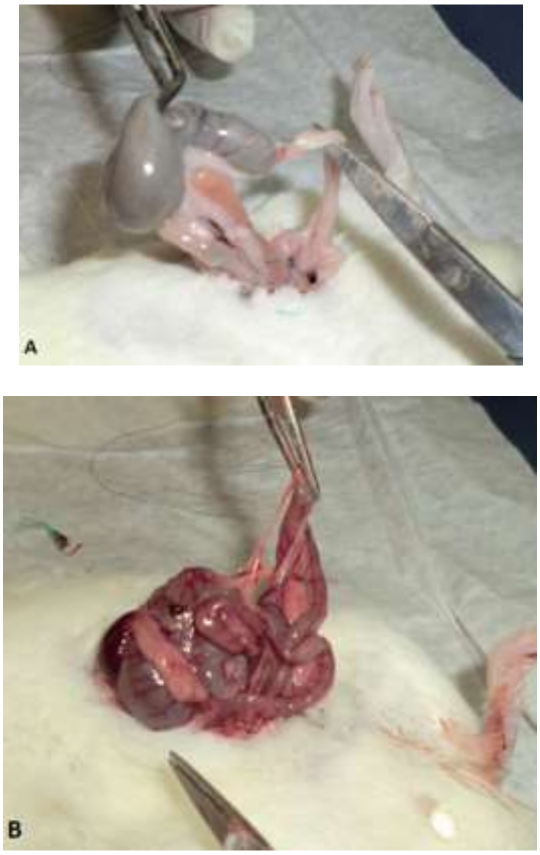

Figure 1. Adhesion formation in cecum.

Table 1. Scoring system for adhesion based on surgeon criterion.

Grade	Nair *et al*., scale
0	Complete absence of adhesions
1	Single band of adhesions, between viscera, or from 1 viscera to abdominal wall
2	Two bands: between viscera, or from viscera to abdominal wall
3	More than 2 bands: between viscera, or from viscera to abdominal wall, or whole of intestines forming a mass without being adherent to abdominal wall
4	Viscera directly adherent to abdominal wall, irrespective of number and extent of adhesive bands

Table 2. Scoring system for adhesion based on pathology criterion.

Pathology criterion	0	1	2	3
Fibrosis	Negative	Partly	Moderate	Severe
Inflammation	Negative	Presence of giant cell, macrophage, lymphocyte and plasma cell	Polymorphonuclear leukocytes and eosinophils	Presence of high inflammatory cells and microabscess
Neovascularization	Negative	1-3 capillaries	4-10 capillaries	>10 capillaries

Group 3, 1 cc hyaluronic acid gel (24mg/ml) was added into the abdomen. In Group 4, a 1 × 1 cm interceed was placed at the injured site of the cecum. In Group 5, 30 mg/kg atorvastatin diluted in 10 cc distilled water was added into the abdomen. The abdominal incision was closed in two layers with a continuous 3-0 silk suture. Eventually, gentamicin was sprayed on sutured place to prevent any possible infections. After 14 days, all animals were sacrificed by an excessive dose of ketamine and xylazine. The abdominal cavity was inspected and the adhesions were graded.

2.3. Evaluation of Adhesion Formation

The adhesions were graded by one expert surgeon, who was blinded to group assignment. The adhesions were scored using the classification reported by Nair *et al*., [15] (Table 1).

2.4. Histologic Examination

A fragment of cecum contained adhesion was evaluated under standard histologic examination. Adhesion-carrying tissues were excised and fixed in formaldehyde solution. Upon dehydration and embedded in paraffin, sections with a thickness of 5 μm were stained with Hematoxylin–Eosin (H & E) stain and examined under a light microscope (Figure 2). Adhesions were graded in all samples based on inflammation, vascularization and fibrosis in granulation sites (Table 2). Normal cell count was graded 0, slight increase in cells was graded 1, marked infiltration was graded 2, and massive infiltration was graded 3 (Figure 2). The histopathological examination of the specimen was performed by a

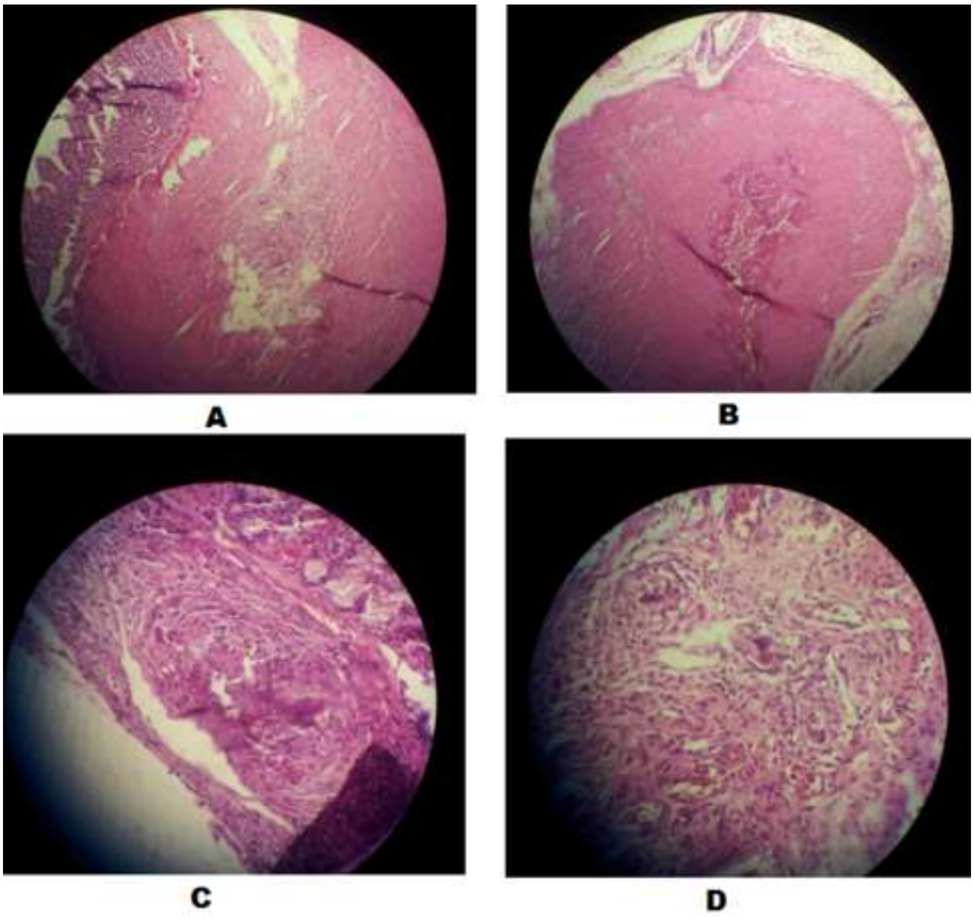

Figure 2. The presence of (A and B) inflammation, (C) fibrosis and (D) neovascularization (HE × 20).

pathologist blinded to the groups. The pathologist was an expert person and a member of pathology department at Baqiyatallah hospital.

2.5. Statistical Analysis

All data are presented as means ±SD. The mean of all findings between the different groups was compared using the One-Way ANOVA: Post Hoc-Tukey test. Data were analyzed using SPSS software (version 21). A p-value of less than 0.05 was considered as significant.

3. Results and Discussion

During the study, two animals with early death were removed from the study. One animal from Group 2 died during anesthetic induction and one animal from Group 4 died on the first postoperative day. On macromorphologic evaluation, the distribution of animals according to adhesions and mean adhesion scores of the groups are shown in Tables 3 and 4, respectively. The mean adhesion score in Group 3 (1.18 ± 1.24) was significantly lower compared to Group 4 (3.43 ± 0.53) (p=0.004). There was no significant difference in mean of adhesion score between the other groups (Table 4).

Table 3. The distribution of rats according to the adhesion score

	Groups					
Adhesion score	**Group 1 Sham**	**Group 2 Control**	**Group 3 HA**	**Group 4 OC**	**Group 5 Atorvastatin**	p-value
0	8	1	3	0	0	0.013
1	0	0	3	0	1	
2	0	1	0	0	1	
3	0	2	2	4	4	
4	0	3	0	3	2	
Total score	0	20	9	24	23	

HA: hyaluronic acid; OC: oxidized cellulose

Table 4. Mean of adhesion score based on the surgeon evaluation

Groups	**Adhesion score**
Group 1 (Sham)	0
Group 2 (Control)	2.86 ± 1.46
Group 3 (HA)	1.18 ± 1.24*
Group 4 (OC)	3.43 ± 0.53
Group 5 (Atorvastatin)	2.88 ± 0.99

HA: hyaluronic acid; OC: oxidized cellulose; *p=0.004 compared to group 4

Table 5. The distribution of rats according to the adhesion score.

	Groups					
Adhesion score	**Group 1 Sham**	**Group 2 Control**	**Group 3 HA**	**Group 4 OC**	**Group 5 Atorvastatin**	**p-value**
0	8	1	1	1	1	0.68
1	0	0	0	0	0	
2	0	0	0	0	0	
3	0	0	2	2	3	
4	0	0	0	0	0	
5	0	0	0	0	0	
6	0	0	0	0	0	
7	0	0	0	0	0	
8	0	0	0	0	0	
9	0	6	5	4	4	
Total score	0	54	51	42	45	

HA: hyaluronic acid; OC: oxidized cellulose

On pathologic examination, the distribution of animals according to adhesions and mean adhesion scores is shown in Tables 5 and 6, respectively. The mean of adhesions total score in all groups was slightly lower compared to the control group (7.71 ± 3.4); however, these differences were not statistically significant. Group 5 had the lowest mean of adhesions total score on pathological examination (Table 6).

The mean of adhesions total score based on both pathologic and surgeon evaluations can be seen in table 7. Although the mean of adhesions total score in all groups was slightly lower than the control group (5.28 ± 3.56), these differences were not significant. Group 3 had the lowest mean of adhesions total score.

Table 6. Mean of adhesion score of the study groups.

Groups	Adhesion score
Group 1 (Sham)	0
Group 2 (Control)	7.71 ± 3.4
Group 3 (HA)	6.37 ± 3.37
Group 4 (OC)	6.0 ± 3.87
Group 5 (Atorvastatin)	5.62 ± 3.73

HA: hyaluronic acid; OC: oxidized cellulose

Table 7. Mean of adhesion total score based on pathologic and surgeon evaluations

Groups	Adhesion score
Group 1 (Sham)	0
Group 2 (Control)	5.28 ± 3.56
Group 3 (HA)	3.82 ± 3.75
Group 4 (OC)	4.71 ± 2.97
Group 5 (Atorvastatin)	4.46 ± 3.32

HA: hyaluronic acid; OC: oxidized cellulose

Recent studies have indicated that more than 95% of patients suffer from adhesions after abdominal surgery [16]. Although adhesions are clinically asymptomatic in most cases, they can be associated with several adverse effects such as intestinal adhesions, chronic pelvic pains, increased surgery time, loss of blood and intestinal injuries during surgery, which subsequently enhance ICU admission and hospitalization time [17]. Since these problems have economic impacts on a society, a better therapeutic method and strategy is needed to prevent intra-abdominal adhesions in patients undergoing abdominal surgery. Currently, there are two strategies for the prevention of adhesions. The first strategy is related to minimizing surgery-induced injuries by diminishing the use of cauterization, laser and retractors, mild touching with tissues and preventing tissue injury or ischemia [12]. The second method including use of membranes and gels as a barrier that isolates injured mesothelial cells areas and causes improvement without adhesions [4]. Drug therapies or use of physical blockers have been frequently recommended for the prevention of abdominal adhesions for several years. Hyaluronic acid, oxidized cellulose and atorvastatin are examples of mechanical blockers and drugs that have various clinical applications.

In this research, the macromorphologic, histologic and pathologic effects of hyaluronic acid, oxidized cellulose and atorvastatin were evaluated on reducing intestinal adhesions after open abdominal surgery in rats. We found that the mean of adhesions total score in atorvastatin group was slightly better than in the control group. Several lines of studies illustrated the positive effect of statins in treatment of adhesions. For example, Aarons *et al*., showed that statins (both atorvastatin and lovastatin) significantly decreased the intraperitoneal adhesions by enhancing the fibrinolytic activity of peritoneal [18]. Nevertheless, they suggested that therapeutic effect of statins in prevention of adhesions in human needs further considerations. Hashemzadeh *et al*., demonstrated that intraperitoneal administration of lovastatin (30mg/kg) in 10cc normal saline caused a significant decrease in the number of sticky bands and severity of intra-abdominal adhesions after laparotomy [19]]. Lalountas *et al*., found that therapeutic effect of intraperitoneal administration of atorvastatin on adhesions formation is significantly higher than seprafilm hyaluronic acid [12]. In another research, these authors showed that statofilm (atorvastatin + carboxymethyl cellulose) had significantly more therapeutic effect in prevention of adhesions formation than seprafilm [20].

In the present study, we observed that the adhesions score in hyaluronic acid was slightly better than that in control; however, this difference was not significant. A large number of studies have considered therapeutic effect of hyaluronic acid against adhesions formation in different types of surgeries. A recent study on 40 guinea pigs has revealed that hyaluronic acid is effective against adhesion formation after abdominal surgery [21]. In another research, Sufiyarov demonstrated that hyaluronic acid film is effective for reducing of peritoneal adhesion in rats [22]. Another previous study indicated that hyaluronic acid decreased adhesions after uterus surgery in rats by making chemical cross-links [4]. Similarly, Johns *et al*., showed that hyaluronic acid is very effective in treatment of adhesion by making Fe^{3+} cross-links [23].

In this study, we observed that adhesions score in oxidized cellulose was slightly lower than that in control group. Gomez *et al*., showed that oxidized cellulose significantly decreased adhesions formation in 20 doges undergone anastomose method by laparoscopic stapler [24]. Interestingly, Marana *et al*., illustrated that oxidized cellulose not only decreased the risk of adhesion and its severity in 29 rabbits after midline laparotomy and standard ovarian surgery, but also it improved reproductive outcomes [25]. A previous investigation reported that oxidized cellulose reduced lesions and adhesions formation in neighboring organs. On the other hand, Arora *et al*., indicated that oxidized cellulose is not effective in treatment of adhesion formation after ovarian surgery in female rabbits [26].

Our data have revealed that adhesions score in hyaluronic acid is significantly better than that in oxidized cellulose. Furthermore, we didn’t find any side effects after drugs therapy which is in agreement with the results obtained from previous research.

4. Conclusion

According to previous accomplished data and our findings, hyaluronic acid, oxidized cellulose, and atorvastatin are effective in treatment and prevention of intestinal adhesions during open abdominal surgery. However, several factors such as type of surgery and single or combinational therapy with these drugs may be important to obtain better results. Although hyaluronic acid was approximately more effective, further studies with larger sample size are suggested to evaluate the combinational therapy of these drugs.

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