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Original Article

The effects of mythomycin C, hylan Gf20 and honey combinations on adhesion formation in laparotomized rats

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Abstract

Aim: Postoperative adhesions are stil unsolved important problems. Some studies have been showed that Mitomycine, Hylan GF-20 and honey can decrease adhesion formation. This study; was planned to aim to compare the adhesion formation effects of Mitomycin C with Hylan GF-20, Mitomycin C with honey and Hylan GF-20 with honey combinations in the laparotomized rats.

Material and Methods: Wistar-Albino 70 malerats, weight range between 180-220 gr, divided into 7 groups. After abrasion formation in the rats' caecum wall and in the peritoneal surface at the localization of right lower quadrant of anterior abdominal wall, Mitomycin C, Hylan GF-20 and honey combinations used and seven days after operation the abdomen was opened for examination.

Results: There were statistically significant differences between control group and the study groups according to adhesion formation scala. In all groups that agents were used adhesion formation was decreased, but when the groups comparing the agents used, there was no statistically significant difference.

Conclusion: Mitomycin C, Hylan GF-20 and Honey usage in the laparotomized rats decrease adhesion formation. But the combinations of these agents do not show any additional effect to decrease adhesion formation.

Keywords: Adhesion; Mitomycin-C; Hylan Gf-20; Honey; Laparotomy; Rats.

INTRODUCTION

Since the initiation of intra-abdominal interventions, postoperative adhesion development has become an important problem. Despite the numerous advances in surgical instrumentation and techniques used to prevent adhesion development, intra-abdominal adhesions are still a major clinical issue. The role of careful surgical technique, less traumatization of tissues during surgery, and well-controlled bleeding control, also known as the Halsted principles, in preventing postoperative adhesions are not the ultimate solution (1). Intra-abdominal adhesions can lead to intestinal obstruction, chronic pelvic pain, infertility, ureteral obstruction, bladder dysfunction, dyspareunia, ineffective intra-peritoneal treatment and difficulty in recurrent operations and morbidity (such as organ perforation). To date, many intra-peritoneal agents have been tried to prevent adhesions. Hylan GF-20 is a liquid-based hyaluronic acid (HA) derivative used in orthopedic surgery to reduce intra-articular adhesions and enhance lubrication on joint surfaces, and has been shown to significantly reduce adhesion development in some studies (2,3). Mitomycin C (MMC) is an anti-metabolic agent that inhibits fibroblast proliferation by inhibiting fibroblastic growth factor and has been shown to significantly reduce postoperative intra-abdominal adhesions in experimental studies (4). Honey has a broad spectrum of actions, such as antifungal, cytostatic, anti-inflammatory, and wound healing enhancement, and has been experimentally shown to significantly reduce the development of postoperative intra-abdominal adhesions (5, 6). The aim of this experimental study was to compare the effects of MMC plus Hylan GF-20, MMC plus honey, and Hylan GF-20 plus honey combinations on adhesion formation in laparotomized rats.

MATERIAL and METHODS

This experimental study was carried out at the Experimental Research Laboratory of Fırat University Faculty of Medicine, following the approval of the Local Ethics Committee with the date and number of 03.02.2005/2/10. There is no informed consent because of the experimental animal based nature of the study. A total of 70 Wistar-

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Albino male rats weighing 180-220 g were used in the study. The rats were kept in cages, five in each group, until the end of the experiment, and standard pellet diet and tap water were used for feeding the rats. The rats were kept under constant temperature and humidity. The feeding of all rats was stopped 12 hours prior to surgery. The subjects were divided into seven groups each consisting of ten rats:

Group 1: Control group; an abrasion was made in the cecum through laparotomy and 5 ml of 0.9% NaCl solution was administered intra-peritoneally.

Group 2: The group in which an abrasion was made in the cecum with laparotomy and

1 mg / kg Mitomycin-C dissolved in 5 ml of 0.9% NaCl was administered intra-peritoneally.

Group 3: The group in which an abrasion was made in the cecum with laparotomy and

Hylan GF-20 (1 cc dose of 4% solution) was administered intra-peritoneally.

Group 4: The group in which an abrasion was made in the cecum with laparotomy and 2 ml of honey solution diluted with 0.9% NaCl in a one-to-one ratio was administered intra-peritoneally to cover the abraded area.

Group 5: The group in which an abrasion was made in the cecum with laparotomy and 1 mg / kg Mitomycin-C dissolved in 5 ml of 0.9% NaCl + Hylan GF-20 (1 cc of 4% solution) was administered intra-peritoneally.

Group 6: The group in which an abrasion was made in the cecum with laparotomy and 1 mg / kg Mitomycin-C dissolved in 5 ml of 0.9% NaCl + 2 ml of honey solution diluted with 0.9% NaCl in a one-to-one ratio was administered intra-peritoneally to cover the abraded area.

Group 7: The group in which an abrasion was made in the cecum with laparotomy and Hylan GF-20 (1 cc of 4% solution) + 2 ml of honey solution diluted with 0.9% NaCl in a one-to-one ratio was administered intra-peritoneally to cover the abraded area.

In order to establish general anesthesia, Ketamine HCL (Ketalar® Flakon, Eczacıbaşı, Istanbul, Turkey) at a concentration of 50 mg / ml and Xylazine HCL 2% (Rhompun® Flakon, Bayer, Leverkusen, Germany) at a concentration of 20 mg / ml, each with a dose of 0.25 ml per 100 gr body weight, were administered intramuscularly at the right hind leg of the rats. After anesthesia induction, the abdomen was shaved and the operation area was cleaned with 10% Povidone Iodine. The intervention site was draped with sterile dress, which only left the incision site open. A 4 cm vertical midline incision was made on the abdominal wall and the skin, subcutaneous tissue, linea alba, and peritoneum were opened and the cecum was removed. A 1 cm^2 serosal area was abraded by brushing with a sponge until petechial hemorrhages occurred. Peritoneal abrasion was also created in a 1 cm^2 area by brushing on the right side of the abdominal wall opposite the cecum. The above-mentioned agents were administered intra-peritoneally as described, and then the abdominal peritoneum, subcutaneous tissue and the skin were closed together with continuous 4/0 silk sutures. Seven days after the operation, another surgeon who was blind to the study groups, opened the abdomen under both rib arches with a reverse U incision (Figure 1) and adhesions were scored into 4 groups according to the severity (Table 1) (7).

Figure 1. The appearance of a rat with a adhesion score of 1 (belonging to Group 3)

Statistical Analysis

The differences between the adhesion scores obtained in each study group were assessed using the Mann-Whitney U test. The data are given as mean ± standard deviation (SD). Analysis of the data was made using the SPSS version 11.0 for Windows. A p value of <0.05 was considered as statistically significant and a p value of <0.001 was considered as strongly significant.

RESULTS

There was a statistically strongly significant difference in adhesion development scores between the control group and the study groups that received intra-peritoneal drug administration. Accordingly, Mitomycin C, Hylan GF-20, honey, Mitomycin C + Hylan GF-20, Mitomycin C + honey, and Hylan GF-20 + honey-administered rats had significantly lower intra-abdominal adhesions development compared to the control group (Table 2).

Table 1. Scoring of abdominal adhesions

Score	state of adhesions
0	• No adhesion
1	• Adhesions that can easily open with fine, avascular, blunt dissection
2	• Limited vascularised adhesions that can open with aggressive blunt dissection
3	• Well vascularised adhesions that can only open with sharp dissection

Table 2. Distribution of adhesion scores in accordance with the groups

	Scores of adhesions			
	score 0	score 1	score 2	score 3
Groups				
Group 1 (n=10)	0	1	4	5
Group 2 (n=10)	6	4	0	0
Group 3 (n=10)	5	4	1	0
Group 4 (n=10)	4	4	2	0
Group 5 (n=10)	8	2	0	0
Group 6 (n=10)	8	2	0	0
Group 7 (n=10)	5	4	1	0

There was no statistically significant difference between the single agent administered groups (Mitomycin C, Hylan GF-20, and honey) in terms of adhesion development score ($p> 0.5$, for all). Accordingly, the single groups did not have superiority over each other in terms of preventing adhesion formation.

There was no statistically significant difference between Mitomycin C and its combinations with Hylan GF-20 and honey (Mitomycin C + Hylan GF-20, and Mitomycin C + honey) in terms of adhesion development score ($p>0.05$, for both). Accordingly, combination with HylanGF-20 or honey did not increase the adhesion-reducing effect of Mitomycin C.

There was no statistically significant difference between honey and its combinations with Mitomycin C and Hylan GF20 (Mitomycin C + honey, and Hylan GF20 + honey) in terms of the adhesion development score ($p>0.05$, for both). Accordingly, a combination with Mitomycin C or Hylan GF-20 did not increase the adhesion-reducing effect of honey.

There was no statistically significant difference between Hylan GF-20 and its combinations with Mitomycin C and honey (Mitomycin C + Hylan GF-20, and Hylan GF-20 + honey) in terms of the adhesion development score ($p>0.05$, and $p = 1.0$, respectively). Accordingly, a combination with Mitomycin C or honey did not increase the adhesion-reducing effect of Hylan GF-20.

There was no statistically significant difference between the combination groups (Mitomycin C + Hylan GF-20, Mitomycin C + honey, and Hylan GF-20 + honey) ($p = 1.0$, $p>0.05$, and $p>0.05$, respectively). Accordingly, combinations of Mitomycin C + Hylan GF-20, Mitomycin C + honey and Hylan GF-20 + honey were not superior to each other in terms of their adhesion-reducing effects.

DISCUSSION

Fibrous adhesions develop as a result of the peritoneal response to injury. An inflammatory reaction begins when the peritoneum becomes exposed to a chemical agent, ischemia or mechanical trauma. The destruction of mast cells and the release of vasoactive amines increase the permeability of blood vessels and stimulate the release of a rich exudate. Coagulum formation is followed by fibrin and fibrin network formation. This fibrin is covered with macrophages, fibroblasts and mesenchymal cells, as a result of which granulation tissue develops. Fibrin cannot be dissolved when there is no peritoneal fibrinolysis activity. Fibrinous adhesions that do not dissolve in more than three days result in fibroblastic transformation and peritoneal adhesion development (8). There are several approaches to prevent adhesion development such as preventing or limiting the initial peritoneal injury, preventing coagulation of the serous exudate, removing or dissolving the accumulated fibrin, preventing fibrin-coated peritoneal surfaces from touching each other until new mesothelial cells are formed, and inhibition of fibroblastic proliferation (4). A number of agents have been used in an attempt to prevent adhesions. The main ones are pharmacological agents (NSAIDs, corticosteroids, antihistamines, progesterone / estrogen, anticoagulants, fibrinolytics, antibiotics) and peritoneal barriers (9). Despite ongoing studies and improvements, the incidence of adhesion development has been reduced, but adhesion development has not been completely prevented.

MMC was obtained from Streptomyces cuspidatus and was initially used as an aminoglycoside antibiotic, and since 1983 it has been used as an antineoplastic agent in humans (10). It acts as an alkylating agent and breaks down DNA cross-links and inhibits protein and RNA synthesis at high concentrations, resulting in antineoplastic effect (11). One of the best known effects of MMC is inhibition of fibroblast proliferation (12). For the last 20 years, the ability of MMC on reducing scar tissue formation has been investigated and it shows this feature by inhibiting fibroblast proliferation. The anti-proliferative effects of MMC in human fibroblast cell cultures have been observed at low concentrations, whereas the fatal effects on these cells occur at high concentrations (10). In our study, the use of intra-peritoneal MMC in laparotomized rats resulted in a strongly significant decrease in adhesion development compared to the control group, and these findings were consistent with the literature (9,13).

HA is a natural glycosaminoglycan with repeating N-acetylglucosamine and D-glucuronic acid units in its structure (14). It is a major component of the extracellular matrix and is found in connective tissue, skin, cartilage, vitreous fluid and synovial fluid. HA is non-immunogenic, non-toxic and a natural bioabsorbable. It is negatively charged and freely soluble at physiological pH, similar to

carboxymethylcellulose. HA covers the serosal surfaces and protects these surfaces from dryness and other types of tissue damage. HA accelerates healing without causing excessive connective tissue growth in many tissues, including the peritoneum. It is thought that organs swim in the intra-peritoneally administered HA solution and that the surface coating property of HA plays a role in its anti-adhesive effect (15-18). Indeed, Reijnen and colleagues demonstrated that 0.4% hyaluronic acid solution significantly reduced the postoperative adhesion development in rats (17). In our study, the use of intra-peritoneal Hylan GF-20 in the laparotomized rats was found to significantly reduce the adhesion formation compared to the control group, which was consistent with previous studies in the literature (2,3).

Honey has been medically used since ancient times. Honey has a broad-spectrum effect such as antifungal, cytostatic, anti-inflammatory and wound healing accelerating effects (5,19,20). Physical properties of honey such as hygroscopicity, low pH and hypertonicity are believed to be responsible for its favorable effects on wound healing (20, 21). Some studies have even suggested that it has additional anti-tumoral and anti-metastatic properties (20,22,23). Caffeic acid, benzoic acid and esters, phenolic acid and esters, flavanoid glycols, wax, inhibin and catalase that honey contains may be responsible for the accelerated wound healing. Inhibin and catalase have been shown to have an effect on epithelial growth (21,24,25). Aysan et al. have shown in an experimental study that intra-peritoneally administered honey significantly reduces the postoperative adhesions. It has been suggested that delayed absorption of honey due to its physical properties (especially hypertonicity) and consequent mechanical interference between surfaces, and the favorable effects of honey on the healing process following peritoneal injury result in significant reduction in postoperative adhesion development (5). In our study, the use of intra-peritoneal honey in laparotomized rats strongly significantly decreased adhesion development compared to the control group. However, Aysan and his colleagues administered 5 ml of pure honey solution intra-peritoneally. In our study, all rats died soon after administration of 5 ml of pure honey solution intra-peritoneally in the first step. Therefore, we used one-to-one diluted honey solution with 0.9% NaCl in our study and administered 2 ml of this solution intra-peritoneally. Since no more rats died after this administration, we carried out the study in this way after exclusion of the previously dead rats. The fatality in the first case may arise from the hygroscopic and hypertonic nature of honey which leads to fluid migration into the peritoneal cavity from the intravascular space and excessive amount of honey administration may play role in the death of rats.

To the best of our knowledge, there is no study investigating the effects of combinations of Mitomycin C + Hylan GF-20, Mitomycin C + honey, and Hylan GF-20 + honey on abdominal adhesion development. In our study, we observed that Mitomycin C, Hylan GF-20 and honey individually or in combination, as Mitomycin C + Hylan GF-20, Mitomycin C + honey, and Hylan GF-20 + honey, reduced the development of abdominal adhesions compared to the control group. However, individual use of substances was not superior to their combined use and neither each individual agent nor combinations were statistically superior to each other in terms of preventing abdominal adhesions. This may remind us that the biochemical mechanisms of substances in preventing adhesion separately are not superior to each other.

CONCLUSION

As a result, intra-peritoneal administration of Mitomycin C, Hylan GF-20 and honey reduce peritoneal adhesion development in laparotomized rats. While each of the combination also reduces abdominal adhesion development, their combination as Mitomycin C + Hylan GF-20, Mitomycin C + honey and Hylan GF-20 + honey does not contribute to their individual adhesion prevention effect.

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