Which Is Most Effective In Prevention Of Postoperative Intraperitoneal Adhesions - Methylene Blue, Low Molecular Weight Heparin Or Vitamin E: An Experimental Study In Rats

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Abstract

Background: Postoperative intraperitoneal adhesions and their complications are still serious problems, which are complicating abdominal surgery.

Aim: To asses the effects of methylene blue, low molecular weight heparin (LMWH) and vitamin E in prevention of postoperative intraperitoneal adhesions. We also aimed to assess which one is the most effective one.

Material and Method: 40 Wistar albino rats were divided into four groups. The first group was the control group; methylene blue was given intraperitoneally to the second group, LMWH to the third group and vitamin E to the fourth group. At the 7th postoperative day, the rats were sacrificed by an overdose of ether, and macroscopic staging for adhesions was performed blindly.

Results: Adhesion formation in group 2, 3 and 4 was less than in the control group and a statistically significant difference was determined in comparison of those groups ($p<0.005$). No statistically significant difference was found at macromorphological staging between groups 2, 3 and 4, although some differences were noted ($p>0.05$).
Conclusion: LMWH and vitamin E with their low prices, easy applications and no toxic and side effects may have an alternative clinical application field in prevention of adhesions.

INTRODUCTION

Abdominal surgery can cause adhesions between tissues and organs. Approximately 93% of the patients who had undergone one or more previous surgeries had intra-abdominal adhesions[1]. Postsurgical adhesions are a consequence resulting when injured tissue surfaces, following incision, cauterization, suturing or other means of trauma, fuse together to form scar tissue. Recently, it was found[2] that all patients who had undergone at least one prior abdominal surgery developed one to more than ten adhesions. Postsurgical adhesions severely affect the quality of life of millions of people worldwide, causing small-bowel obstruction[1], difficult reoperative surgery[3], chronic abdominal and pelvic pain[4], and female infertility[5].

Reoperating through a previous wound can be extremely difficult, risky, and potentially dangerous. Also, adhesiolysis extends operating time, anesthesia, and recovery time and causes additional risks to the patient such as blood loss, visceral damage including injury to the bladder, enterocutaneous fistulas, and resection of damaged bowel[6].

In this experimental study, we aspired to evaluate the effects of methylene blue (MB), low molecular weight heparin (LMWH) and vitamin E, which effect by different mechanisms but are applied from the same way, in prevention of postoperative intraperitoneal adhesions. All of these three substances are cheap and easy to apply; that is why we selected them. We also aimed to assess which one is the most effective one.

MATERIALS AND METHODS

The Ethics Committee of our center approved the

experimental procedures in this study. All of the guiding principles in the care and use of laboratory animals were strictly adhered to throughout the entire study.

Forty Wistar female rats, weighing between 190 and 210g were housed in a climate controlled (relative humidity of 30-70% and temperature of 20°C to 24°C) animal-care facility, with a 12-hour light/dark cycle. The animals were provided with standard rodent chow and water ad libitum.

The rats were anesthetized with intramuscular injection of ketamine hydrochlorur (40mg/kg). The surgical field was shaved and prepared with 1% antiseptic povidine-iodine solution and a 3cm incision was made along the abdominal midline. Punctate serosal hemorrhagies were created by scraping the anterior wall of the caecum with mesh gauze. This is a standard accepted procedure. Prior to closure of the abdominal incision, animals were randomly assigned to intraperitoneal administration of 2mL of either saline (n=10) (as control group), 1% Methylene blue solution (n=10), LMWH (100U a x a /kg) (Clexane®, Aventis Pharma – France) (n=10), or vitamin E (10mg/kg in 2ml sterilized pure olive oil) (n=10). The abdominal incision was subsequently closed in two layers; muscle layer with a continuous 4-0 polyglycolic acid suture and skin with 4-0 polypropylene suture one by one. At the 6th postoperative hour, feeding of the animals was started.

The animals were allowed to resume their diet until the 7th postoperative day, when they were sacrificed after exposure to an overdose of ether. The intraabdominal cavity was inspected through a U-shaped incision of the anterior abdominal wall, which was retracted caudalad, providing maximal exposure.

Qualitative and quantitative grading of adhesions was performed according to the scoring system which has created by Blauer et al.$_7$ (Table 1). The macroscopic staging of adhesions was performed by another surgeon blindly.

The results of the macromorphological staging were statistically analyzed with Kruskall-Wallis test.

Figure 1

Table 1: Adhesion Scoring System

Grade	*Description*
0	No Adhesions
1	Thin or narrow, easily separable adhesions
2	Thick adhesions limited to one area
3	Thick and widespread adhesions
4	Thick and widespread adhesions, plus adhesions of viscera to anterior and/or posterior abdominal wall

RESULTS

The intra-abdominal adhesions and adhesion scores are summarized in table 2. There were statistically significant differences between the control group and methylene blue, LMWH and vitamin E groups individually ($p<0.05$). No statistically significant differences were found when the groups 2, 3 and 4 were compared with each other ($p>0.05$).

All results of the groups can be seen in table 2. There were neither complications nor mortality during the study. The pictures of the adhesion levels can be seen in Figure 1.

Figure 2

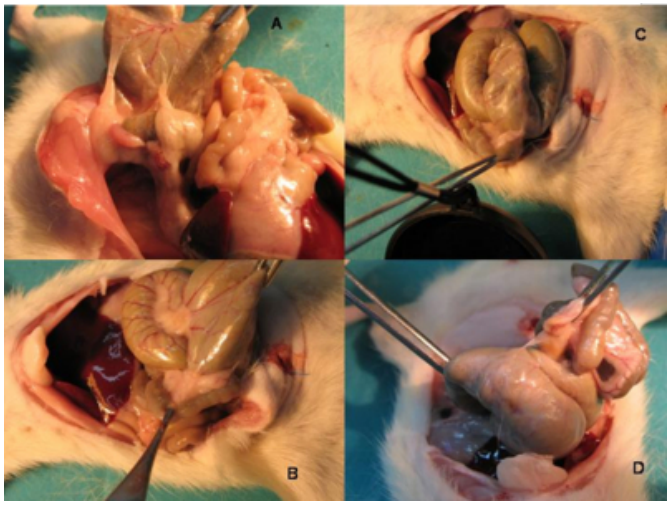
Table 2: Adhesion Results of the Groups

GROUPS	N	SCORE					AVERAGE
		0	1	2	3	4	
Control	10	0	0	4	5	1	3±0.67
M. Blue	10	2	4	3	1	0	1±0.94
LMWH	10	3	2	5	0	0	1.5±0.91
Vitamin E	10	1	5	3	1	0	1±0.84

All groups showed a statistically significant difference to the control group. Also, we found that there is no statistically significant difference between MB, LMWH and vitamin E according to prevention of intraperitoneal adhesions.

Figure 3

Figure 1: A) Level-4 peritoneal adhesion, a rat from control group. Level-3 peritoneal adhesion, a rat from methylene blue group. Level-2 peritoneal adhesion, a rat from LMWH group. Level-1 peritoneal adhesion, a rat from LMWH group.



DISCUSSION

In our study, we used the optimal doses and the same preparation style which were reported before. Thus, we did not have a Sham group and a control group using olive oil only. These three substances were studied individually by

other authors. Ours primary aim was to evaluate the differences between these three cheap and easy-to-apply substances. But we did not find any statistically significant difference between them.

Numerous mediators of inflammation, such as arachidonic acid, cytokines, nitric oxide, and oxygen derived free radicals might participate in postoperative formation of adhesions. The antioxidative properties of vitamin E[8], and other compounds such as glucocorticoids and aspirin, are believed to be related to their possible ability to prevent adhesions. This is the first study in which these three effective and cheap substances were evaluated for prevention of postoperative adhesions.

Vitamin E theoretically has interesting biologic properties and actions for the prevention of peritoneal adhesions. In vitro studies have shown that vitamin E has antioxidant, anti-inflammatory, anticoagulant, and antifibroblastic effects, and decreases collagen production. It has been successfully used, administered by the oral route, intramuscularly and especially intraperitoneally; diluted in olive oil, in animal models. Vitamin E was found effective for reducing adhesion formation by some authors[9,10,11]. We found vitamin E effective at preventing intraperitoneal adhesions ($p<0.05$).

Methylene blue is known to inhibit the generation of oxygen radicals such as superoxide by competing with oxygen for the transfer of electrons from flavor-enzymes, primarily xanthine oxidase[12, 13]. This low molecular weight, partially liposoluble vital dye, is also a known inhibitor of guanylate cyclase. It organizes the smooth muscle relaxation effects of nitric oxide, blocking its activation by blocking NO binding sites of guanylate cyclase[14,15, 16]. In recent studies, it has been suggested that intraperitoneal application of methylene blue can be used as an effective agent in the prevention of postoperative adhesions[17,18,19]. We also found that methylene blue prevents postsurgical adhesions ($p<0.05$). Dinc et al. suggested that methylene blue prevents peritoneal adhesions but causes a significant impairment of anastomotic bursting pressure during the early phase of the wound healing process by its transient inhibitory effect on the nitric oxide pathway[20].

LMWH reduces peritoneal adhesion by increasing the fibrinolysis due to serine esterase activity[21]. Many authors suggested that LMWH prevents postsurgical intraperitoneal adhesions when administered either subcutaneously or intraperitoneally[22,23]. We also found that LMWH prevents postsurgical adhesions ($p<0.05$).

Neither toxic nor side effects were reported due to any of these substances. We also did not observe any of them.

Numerous attempts have been made to prevent or reduce the incidence of peritoneal adhesions, but with limited success[24,25]. These have included removal of fibrinous exudates by peritoneal lavage with or without the use of various proteolytic enzymes, prevention of fibrin deposition using anticoagulants and anti-inflammatory agents, separation of surfaces by such methods as simulation of peristalsis with prostigmine to prevent prolonged contact between adjacent loops of intestine, use of substances such as olive oil or liquid paraffin, which prevent adhesions formation by limiting tissue apposition during the initial phases of peritoneal repair, inhibition of fibroblastic proliferation with antihistamines, steroids, and cytotoxic agents, and enhancement of peritoneal fibrinolytic activity, which decreases after local peritoneal insult, with application of recombinant tissue plasminogen activator[26].

Experimental studies have demonstrated that covering lesions of the parietal peritoneum with microsurgically applied autologous peritoneal transplants can completely prevent severe adhesion formation[27]. The advantage of a synthetic barrier is that the material does not need to be obtained surgically and can be cut to size outside of the abdomen and then applied without sutures[27].

CONCLUSION

A multifactorial approach including minimizing tissue injury, prophylactic antibiotic usage to reduce infectious morbidity, and biochemical agents with or without biomechanical barriers will reduce amount and severity of adhesions. This was a hopeful study with LMWH, methylene blue and vitamin E. All of them have a good cost/benefit ratio, easy applications and no toxic and side effects and may have an alternative clinical application field in prevention of adhesions. They have no advantage over each other as for prevention of postoperative adhesions. However, researches are needed to establish safety and effectiveness of all these substances in human subjects.

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