EVALUATION OF CLEMASTINE EFFECT ON POSTSURGICAL INTRAABDOMINAL ADHESIONS IN RATS

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Abstract: This study is undertaken to examine the effect of clemastine in prevention of intra-abdominal adhesions after laparatomy in rats. It was a case-control experimental clinical trial. The sample included 60 healthy, male rats that were divided into two equal treated and control groups randomly. The rats, used for experiment, were from the same generation and the same age weighing about 215 ± 20 g. Each rat was anesthetized with ketamine hydrochloride (5mg/kg). Using sterile technique, the abdominal cavity was entered via a 4 cm, vertical midline incision. Five longitudinal incisions each of them 1 cm in length were incised in the internal aspect of their right abdominal wall and a 1 cm² piece of peritoneum was excised from the left side. Concurrently, clemastine (25µg/kg) was given intraperitoneally to the treated group. Two weeks later, they had a second laparatomy for assessing the rate, grade and pathologic evaluation of adhesions. The results showed that the number of adhesion bands and their grading were less in the treated group. The difference between treated and control groups was statistically significant (P<0.0001). The average grading of intra-abdominal adhesions in the treated group was 1.10000 ± 1.0939 and corresponding figure for the control group was 2.40000 ± 1.2484. There was a positive and statistically significant correlation between the number and the grade of adhesions (rhos = 0.457, P<0.0001). Clemastine fumarate effectively decreased the number and the degree of adhesion bands.

Key words: Post-surgical adhesion, Clemastine, Laparatomy.

INTRODUCTION

Postsurgical adhesions occur commonly after surgical procedures and are the source of substantial postoperative morbidity. No preventive or prophylactic regimen against adhesions has proven to be successful. The basic mechanisms causing adhesion formation has not been elucidated fully [1]. More than 3.3% of all laparatomies are due to adhesions [2]. Sixty seven percent of patients with a history of abdominal surgery have problem of adhesions some day in their life; 3-8% of them will proceed to relaparatomy [2].

Pelvic adhesions occur in 55-95% of women with laparatomy. Special surgeries with higher incidence of adhesion formation include: appendectomy, intestinal surgeries, pelvic surgeries (uterine, fallopian and ovarian surgeries). Adhesions can cause bowel obstruction, chronic abdominal or pelvic pain, and infertility [3]. Adhesions have been the most common (40-50%) cause of chronic pelvic pain [4,5]. Furthermore, adhesions have been responsible for a great number (40-60%) of morbidities. In 1996 in United States alone more than 3.2 million dollars were spent for the treatment of adhesions [4,5].

Peritoneal adhesions are fibrous bands of tissues formed between organs that are normally separated and/or between organs and the internal body wall. Most intra-abdominal adhesions are a result of peritoneal injury, either by a prior surgical procedure
or due to intra-abdominal infection. Adhesions form when the peritoneal surface is damaged due to surgery, thermal or ischemic injury, inflammation or foreign body reaction. The injury disrupts the protective mesothelial cell layer lining the peritoneal cavity and the underlying connective tissue. The injury elicits an inflammatory response consisting of hyperemia, fluid exudation release and activation of white red blood cells and platelet in the peritoneal cavity, activation of inflammatory cytokines, and the onset of coagulation and complement cascades [7]. Histamine, released from mast cells, causes vasodilatation and extravasation of an exudate rich of proteins responsible for adhesion formation [5,6]. Fibrin deposition occurs between the damaged but opposed serosal surfaces. During normal repair, fibrin is principally degraded by the fibrinolytic protease plasmin, which is derived from inactive plaminogen. Fibrinolytic activity in peritoneal fluid is reduced after abdominal surgery [7]. Antihistamines inhibit its function and, reduce the increased vascular permeability caused by histamine. In this study it is presumed that histamine release hang-up and the inhibition of its function with antihistamines reduce the adhesion formation. We have chosen clemastine, as an antihistaminic drug, to asess its effect on decreasing of post-surgical abdominal adhesion formation.

MATERIALS AND METHODS

Healthy 60 male rats (from the same generation and the same age), weighing 215 ± 20 g were selected. The animals were divided into two groups containing 30 rats each group and maintained in the animal house of Isfahan college of Medicine, Iran. The one group of animals was marked with red dye and considered as control group. Each rat was anesthetized with ketamine hydrochloride (5mg/kg). Before incision, the abdomen was shaved and prepared with povidine iodine solution. The surgical gloves were cleaned by sterile normal saline. Using sterile technique, the abdominal cavity was entered via a 4cm, vertical midline incision. Five longitudinal incisions (each of them 1cm in length) were incised in the internal aspect of their right abdominal wall and a 1 cm² piece of peritoneum was excised from the left side. Concurrently, clemastine (25µg/kg) was given intraperitonealy to the treated group only.

After operation abdominal wall muscles were closed by chromic and the skin by silk sutures. After 14 days rats had a second laparatomy with the same anesthesia and the same incision for assessing the rate, grade and pathologic evaluation of adhesions. The grade of adhesions were scored by a double blind method (the technician was not aware of the method of injecting drugs or the either of the groups) and recorded in the check lists. The scoring of the grade of adhesion bands were done similar to those used by Avsar et al. [8]. i.e., i). Without adhesion bands, ii). A fine adhesion band that lysis simply, iii). A coarse adhesion band in one site, iv). diffuse adhesion bands, and v). fusion of intra-abdominal organs.

Statistical analysis: Variance, Kruskal and Mannwhitney tests were used for the statistical analysis. The correlation of the number and the grade of adhesion bands were analyzed by using Mann Whitney test and Spearman’s rho matrix. The calculations were done using SPSS 9.5.

RESULTS

In the treated group 10 rats did not produce any adhesion, but in the control group the corresponding figure was only two (Fig. 1). The difference in the number of adhesion bands in two groups was statistically significant (P<0.0001). The average number of adhesion bands in the group receiving clemastine (0.7333 ± 0.5843) was significantly less than the control group (1.5667 ± 0.9714) (P<0.0001). Among the treated rats, two fell in the fourth grading group but none in third grading group. The corresponding figures for the control rats were eight and three (Fig. 2). The grade of intra-abdominal adhesions in the treated group (with average of 1.1
± 1.09) was less than control group (with average of 2.4 ± 1.25). This difference was statistically significant (P<0.0001). The results showed a positive and statistically significant correlation between the number of adhesion bands and the grade of adhesions (P<0.0001).

DISCUSSION

Adhesion bands are fibroblastic proliferation of collagen fibers and some degree of inflammation containing lymphoplasma and polymorphonuclear cells [9]. In an attempt to prevent postoperative adhesion formation a wide variety of surgical adjuvants have been used. Miligan and Raftery [9] showed that adhesion formation follows the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation. It is possible to reduce adhesion formation with various precautions or chemicals at each of these steps. The different treatment modalities fall into five categories, first described by Boys [10] as the five fundamental attacks directed towards the prevention of the adhesions. These attacks are intended (i) to limit or prevent initial peritoneal injury, (ii) to prevent coagulation of the serous exudates, (iii) to remove or dissolve the deposited fibrin, (iv) to keep apart the fibrin-coated peritoneal surfaces until mesothelization has occurred and (v) to inhibit fibroblastic proliferation once it is established. None of these different treatment modalities has prevented the problem entirely, therefore, studies to find safe and effective agents are continuing [10].

A number of inflammatory cells, such as mast cells, eosinophils, macrophages, and neutrophils, are able to directly influence the functional properties of some of the fibroblast and myofibroblast [11]. Among these, the mast cells are recognized as key cells of allergy and have been implicated in a number of chronic inflammatory diseases such as scleroderma, chronic graft-versus-host disease, idiopathic lung fibrotic disease and liver cirrhosis. In addition, mast cells have been attributed a role in peritoneal adhesions in a rat model in which their inhibition was found to be effective in delaying or attenuating adhesion formation [11]. Mast cells, by the activity of specific mediators and of cytokines, can induce proliferation and modulate collagen synthesis in fibroblasts obtained from skin and lung tissues. Additionally, recent observations indicate that mast cells have the ability in normal and pathologic human tissues to directly produce type VIII collagen. Murine mast cell lines and primary mast cells can synthesize basement membrane components such as laminin and collagen type IV [11].

It has also been suggested that histamine is partially responsible for the mast cell enhancing effect on fibroblast migration and proliferation [12] and thereby can exacerbate adhesions formation. Mast cell derived vasoactive and pro-inflammatory mediators, particularly histamine, might contribute to local tissue damage and multiorgan dysfunction induced by intestinal ischemia/reperfusion and it has been shown that ketotifen (an antihistamine drug) is a powerful inhibitor of ischemia/reperfusion-induced leukocyte adhesion and can prevent localized and reduce remote organ damage after intestinal ischemia/reperfusion injury [13].

Intra-abdominal adhesions are one of the major clinical problems of general surgery. In 1996, there was a morbidity of about 40-60% following adhesion bands that is infertility and chronic pelvic pain [3]. Many attempts have been done to decrease the fibroproliferative reactions responsible for adhesion bands. Swolin et al. [14] did not find any statistically significant difference between the monkeys received corticosteroids and their control. In another study Avsar et al. [8] showed that corticosteroids together with antihistamines can be helpful in decreasing adhesion bands by reducing mast cell histamine release. Steinleitner et al. [15] reported that subcutaneous verapamil, diltiazem and nifedipine were also successful in animal models. Intraperitoneal administration of methylene blue was found to decrease the rate of post surgical adhesion formation in rabbits [16]. Likewise, melatonin was found to be effective in preventing post-surgical adhesions [17].

Figure 2. Distribution of the grade of intra-abdominal adhesions in the treated and control groups.
laxipafant plays a role in the prevention of adhesion formation [18] and low dose aspirin prevents the adhesion formation in a rodent model [19].

In present investigation clemastine was used intraperitoneally and after 2 weeks the animals had a second laparatomy for determining the grade, rate and pathologic difference (not presented here) of adhesions in the treated and control groups. Study also shows a statistically significant difference in the rate and grade of adhesions between the treated and the control groups. From over all study it is concluded that clemastine, an antihistaminic drug, can help in decreasing intra-abdominal adhesions postoperatively. Further investigations are suggested to be carried out as well.

REFERENCES