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The comparison of diphenhydramine HCl and Nedocromil sodium in prevention of abdominal postoperative adhesion formation in rat models: An experimental study

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KEYWORDS

Adhesion band;
Postoperative;
Intraperitoneal;
Prevention;
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Nedocromil sodium

Abstract *Background:* The purpose of this study was to determine the effects of diphenhydramine HCl and Nedocromil sodium for the prevention of postsurgical adhesion formation in rat model.

Methods: Sixty adult female rats were anesthetized by 5 mg/kg ketamine hydrochloride. After opening the abdominal wall, a 2 cm² peritoneal layer was excised from the left abdominal wall and 10 longitudinal incisions of 2 to 3 cm in length were made on the right parietal peritoneum. The abdominal wall was closed with 4/0 atraumatic continuous nylon sutures. Group I was the control group, group II was given 10 mg/kg diphenhydramine HCl, group III was given 100 mg/kg Nedocromil sodium, and group IV was administered both drugs in the above doses. All the drugs were instilled into the peritoneal cavity after abdominal closure except Nedocromil sodium which was administered in two separate doses 30 min before surgery and just after abdominal closure. Relaparotomy was performed 2 weeks after the initial surgery and abdominal adhesions were scored. Kruskal–Wallis and Mann–Whitney *U*-test were used for the statistical evaluation. *Results:* The mean ± S.D. (median) of adhesion scores were 2.5 ± 0.90 (2.0), 1.58 ± 0.99 (1.0), 0.92 ± 0.86 (1.0) and 1.75 ± 0.75 (2.0) in group I, II, III and IV, respectively. There were significant differences between the scores of groups I and II ($P = 0.033$), groups I and III ($P < 0.001$), and groups I and IV ($P = 0.033$).

Conclusion: Both diphenhydramine HCl and Nedocromil sodium reduced postoperative abdominal adhesions separately and in combination with each other in our study. Average score of adhesion formation was lowest in the group that was administered Nedocromil sodium. More research is needed in order to discover any positive effect of these drugs as antiadhesive agents in humans. © 2007 Surgical Associates Ltd. Published by Elsevier Ltd. All rights reserved.

Introduction

Fibrous adhesions within the peritoneal cavity are a major clinical problem.¹ Small-bowel obstruction, infertility, chronic abdominal and pelvic pain, and difficult reoperative

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surgery are the most common consequences of peritoneal adhesions.² After laparotomy approximately 95% of patients are shown to have adhesions at subsequent surgery.¹ Peritoneal adhesions account for more than 50%³–60%⁴ of small bowel obstruction cases. Up to 1% of all adult general surgery admission is due to adhesions and, currently, up to 3.3% of all laparotomies are performed for adhesion-related bowel obstruction. In the USA in 1988, costs for abdominal adhesiolysis mounted to nearly \$1.2 billion.⁵

Several mechanisms have been proposed to explain the development of inflammatory and postoperative adhesions, and many factors have been described. Injuries on peritoneal surfaces and existence of ischemic lesions play an important role in abdominal adhesions. Foreign bodies such as talc powder and suture materials, infections and intestinal leakage are also important.⁶ Mast cells have been shown to be involved in peritoneal inflammation that results from either immunologic stimulation or chemical irritation.⁷ Histamine, which is released by mast cells immediately after injuries in peritoneal surfaces, causes vasodilatation and protein enriched exudate. These exudates constitute the base of adhesions.⁶ It has been argued that blocking histamine release and preventing its effects by using antihistamine agents and mast cell stabilizers reduces or eliminates peritoneal adhesion, which still constitute serious surgical problems.^{6–9}

This study was carried out to investigate separate and combined effects of diphenhydramine HCl (an antihistamine agent) and Nedocromil sodium (a mast cell stabilizer) on the prevention of peritoneal adhesions.

Materials and methods

This study was performed in Experimental Animal Laboratory Center, Isfahan University of Medical Science, Isfahan, Iran, with the permission of the Research Committee of Isfahan University of Medical Science (No. 81077) and with the approval of the ethics committee in research of Isfahan University of Medical Science.

Sixty adult female rats weighing 200–220 g were used in this study. The animals were randomly divided into four groups, each including 15 rats. All rats were fed standard rat diet.

The animals were anesthetized by administration of 5 mg/kg ketamine hydrochloride subcutaneously. After the abdomen had been shaved and cleaned with povidone–iodine solution, laparotomy was performed via lower abdominal incision of approximately 3 cm in length. Ten longitudinal incisions of 2 to 3 cm in length were made on the peritoneal surface of the right abdominal wall, and a peritoneal section of 2 cm² was excised from the inside of the left abdominal wall. The abdominal wall was closed with 4/0 atraumatic continuous nylon suture.

The first group (group I) served as the control and was given 1 ml normal saline as placebo. Groups II, III and IV constituted the experimental groups. In group II, 10 mg/kg diphenhydramine HCl was instilled into the peritoneal cavity after abdominal closure. The rats in group III had received an intraperitoneal injection of Nedocromil sodium at a dose of 100 mg/kg 30 min before laparotomy and a dose of 100 mg/kg just after abdominal closure. The rats in group

IV received both 10 mg/kg diphenhydramine HCl after abdominal closure and 100 mg/kg Nedocromil sodium intraperitoneally 30 min before laparotomy and just after abdominal closure. Each compound was dissolved in 1 ml of saline to achieve concentrations of 10 mg/kg diphenhydramine HCl and 100 mg/kg Nedocromil sodium.

After the operation, all rats were placed in their cages and allowed tap water and rat pellets ad libitum for 14 days. Relaparotomy was performed 2 weeks after the initial surgery. The subjects were reanesthetized with 5 mg/kg ketamine and the abdomen was opened from the previous incision line. Left and right abdominal walls and the incision line were investigated with regard to formation of adhesions. The adhesions were scored according to the scoring method developed by Ahmet Canbaz et al.¹⁰ (Table 1). The scoring was done by one of the two calibrated colleagues who did not know to which group the animal was assigned. The average adhesion scores among the four groups were compared. All values were expressed as the mean \pm standard deviation. Statistical evaluation was made with SPSS 9.0 version software. Although we used a Mann–Whitney test to compare scores between groups, Kruskal–Wallis test was carried out compare scores of rats in the four groups, because of abnormal distribution of scores according to the Shapiro–Wilk test. Results were accepted to be significant if the *P* value was less than 0.05.

Results

Unfortunately, 11 rats expired after administration of ketamine HCl and during the postoperative period. The number of formations and grades of abdominal adhesions in the remaining 49 rats are presented in Table 2. The mean \pm S.D. (median) of adhesion scores were 2.5 ± 0.90 (2.0), 1.58 ± 0.99 (1.0), 0.92 ± 0.86 (1.0) and 1.75 ± 0.75 (2.0) in groups I (control), II (diphenhydramine HCl), III (Nedocromil sodium) and IV (diphenhydramine HCl and Nedocromil sodium), respectively. It was found that frequencies and grades of adhesions in the groups that had been administered the drugs decreased significantly in comparison with the control group. The adhesion scores of groups II, III and IV were significantly lower than that of group I ($P = 0.033$, $P < 0.001$ and $P = 0.033$, respectively).

In addition, as the data are not distributed normally additional analysis was done using the chi-square test. Standardized score was calculated by dividing each case's adhesion score by Overall median of cases' adhesion scores (2.0). The mean of these new scores was calculated as 0.83 ± 0.51 and used as a cutoff point to categorize cases. All the cases were divided into two groups according to

Table 1 Modified adhesion scoring scale

Score	Number of adhesions
0	No visible adhesion
1	1 thin, easily separable, avascular adhesion
2	2 thin, easily separable, avascular adhesion
3	3 thin, easily separable, avascular adhesion
4	>3 thin, easily separable, avascular adhesion or vascularized or diffuse adhesions

Table 2 Frequencies of adhesion scores among groups

Adhesion score	Group 1 (n)	Group 2 (n)	Group 3 (n)	Group 4 (n)
0	0	1	4	0
1	1	6	7	5
2	6	2	1	5
3	3	3	1	2
4	2	0	0	0
Total	12	12	13	12

standardized scores ≤ 0.8 or > 0.8 . Frequency of subjects in the group with the lower standardized score (≤ 0.8) was significantly more than the other group in all three intervention groups as compared to the placebo group ($P = 0.002$, Table 3).

Discussion

In this study, induced peritoneal injury was taken as a model, and treatment of pathophysiologic events developing upon peritoneal injury was planned.

In experimental studies, wound fibrinolytic activity has been found to be minimal 3 days after injury. Fibrinolytic activity increases to supernormal levels 8 days after injury. With hypoxia the fibrous adhesions are invaded by fibroblasts and there is stimulation of angiogenesis and collagen synthesis. Fully developed fibrous adhesions are seen at 10 days and become maximal 2 to 3 weeks after peritoneal injury.¹¹

To date, no method of adhesion prevention has been proven in a clinical model and most surgeons rely on strict adherence to Halsted's principles of meticulous tissue handling and the prevention of infection.⁷ Attempts to modulate the fibroproliferative inflammatory response have included the use of steroids and other anti-inflammatory agents, cytotoxic drugs, and antihistamines. The anti-inflammatory and cytotoxic agents decrease the number of adhesions, but at the expense of increased morbidity secondary to delayed wound healing and increased susceptibility to infectious complications.⁹ The mechanism of mast cell involvement in adhesion formation is unclear. Intestinal mast cells release a large number of inflammatory mediators, including histamine, serotonin, lysosomal enzymes, chemotactic factors, cytokines, and oxygen free radicals.⁷ Antihistamines have been used with some success to block release of histamine from mast cells and thereby after the local vasoactive response.⁶⁻⁹

Table 3 Frequency of subject with standardized scores ≤ 0.8 or more

Group	Frequency of cases (%) with standardized score ≤ 0.8	Frequency of cases with standardized score > 0.8
Group I	1 (8.3%)	11 (91.7%)
Group II	7 (58.3%)	5 (41.7%)
Group III	11 (84.6%)	2 (15.4%)
Group IV	5 (41.7%)	7 (58.3%)

Several treatment modalities and agents have been proposed along with protective measures to prevent postoperative abdominal adhesions.^{6,9,12} Agents used for this purpose may be divided mainly into two groups having local and systemic effects.¹² Locally applied agents prevent formation of adhesion by blocking the contact between peritoneal surfaces or by their fibrinolytic effects. Much research has been performed to investigate the role of local barriers in prevention of adhesion formation and the results have shown that these kinds of barriers are useful in preventing adhesion formation.^{13,14} Such barriers are expensive and thus are not accessible to less developed countries.

Administration of locally anti-inflammatory and antihistamine agents have been conducted in much research. Some researchers showed that this method for prevention of adhesion formation is useful in some cases.^{6,7,8,10,15} Systemic agents can be used to prevent adhesion formation. Authors such as Avsar et al.⁶ performed their research according to the hypothesis that systemic agents have more complex effects in the prevention of adhesions. In our study we tried to use antihistamine agents locally and we proposed that locally used agents may have more marked effects due to direct contact with the injured lesions and peritoneal surfaces.

Avsar et al.⁶ showed in their study that diphenhydramine HCl is useful in adhesion formation prevention but they found that the diphenhydramine HCl effect is not superior to that of methylprednisolone. In our study, diphenhydramine HCl administration after surgery was also effective in the prevention of adhesion formation.

Nedocromil sodium is a pyranoquinolone derivative that has been shown to inhibit the activation of several inflammatory cell types, including eosinophils, monocytes, macrophages, platelets, and mast cells in vitro. In vivo study of Nedocromil sodium has demonstrated that this drug prevents anaphylactic bronchoconstriction and bronchial hyperreactivity, and attenuates the increases in vascular permeability and cellular infiltration of the epithelium. More recent work has demonstrated that Nedocromil sodium blocks the chloride channel in several cell types, including mast cells, epithelial cells, and neurons.¹⁶ Administration of Nedocromil sodium for the prevention of postoperative adhesion formation has been evaluated by Liebman et al.⁷ The application of Nedocromil sodium was associated with a significant decrease in the adhesion score when compared with saline. In our study we instilled the same dose of Nedocromil sodium which was used in Liebman's study. The application of Nedocromil sodium with this dose and route of administration significantly decreased the number of adhesions formed when compared to the control group. Also, average adhesion scores were lowest in this group and as a result we came to the conclusion that Nedocromil sodium is more effective in prevention of adhesion formation than saline and diphenhydramine HCl. In addition, results in the group with combined diphenhydramine HCl and Nedocromil sodium were no better than the groups receiving each of these agents separately. While it has been proposed that antihistamines may be used as antiadhesives, the literature does not contain a sufficient number of studies on this subject. Also, antihistamines have the advantage that they have no side effects, they do not have an

adverse effect on injury healing, and they are widely available and inexpensive.

We conclude that diphenhydramine HCl (as an antihistamine agent) and Nedocromil sodium (as a mast cell stabilizer), separately or in combination in abdominal operations, will reduce the rate of postoperative adhesions and related complications. The results of our study indicate that Nedocromil sodium can reduce adhesion formation after abdominal surgery more than diphenhydramine HCl and saline. We believe that these drugs, especially Nedocromil sodium, will have an important role in the prevention of peritoneal adhesions. More research projects must be performed in order to investigate efficacy and safety of these agents in the prevention of adhesion formation.

Conflicts of interest

None.

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Ethical approval

This study was performed in Experimental Animal Lab. Centre, Isfahan University of Medical Science, Isfahan, Iran, with the permission of the Research Committee of Isfahan University of Medical Science and approval of ethics committee in research of Isfahan University of Medical Science.

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