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ORIGINAL ARTICLE

Effect of Combined Therapy with Heparin, Clindamycin and Cefepime on the Prevention of Intra-abdominal Adhesion in Female Rat

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ABSTRACT

Every day, many abdomino-pelvic surgeries are performed around the world. Peritoneal adhesion is one of the most common complications of such surgeries. Many drugs have been suggested to prevent adhesion. It seems that heparin, by preventing fibrin formation, and antibiotics by preventing inflammation would decrease the incidence and severity of adhesion. The aim of this study was to determine the effect of a combined therapy with heparin, clindamycin and cefepime on the prevention of intra-abdominal adhesion in a female rat. In this experimental study, 80 female rats were divided into eight groups including control group, heparin, cefepime, clindamycin, heparin plus cefepime, heparin plus clindamycin, cefepime plus clindamycin, heparin plus cefepime plus clindamycin. After shaving and disinfecting the abdominal region, abdomen opened and multiple scratches were made in the peritoneum and then the abdomen was closed. After two weeks from the surgery, a relaparotomy was performed and a sample of the peritoneum was sent to the laboratory for adhesion grading and histopathological examination. Data was analyzed using SPSS software. According to our findings, the rats of the heparin plus cefepime plus clindamycin group showed a significantly lower incidence and severity of adherence, fibrosis, inflammation and vascular proliferation after surgery in comparison to other groups. The combination of drugs, such as heparin plus cefepime plus clindamycin has an important effect in preventing abdominal adhesions.

Keywords: *Abdominal adhesion, Heparin, Antibiotics, Female Rat*

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INTRODUCTION

Adhesion is one of the modern surgery problems."Adhere" means adhesion or connection with together، in fact it is a process of elimination or impairment of physiological repair of peritoneum, which may be similar to thin palate or rubber bands [1]. Every day around the world Multiple surgeries on the abdomen and pelvic done for various reasons such as appendicitis, cholecystitis, diagnostic laparotomy, tumor and etc [2]. Peritoneal adhesion is one of the common complications of these surgeries [2-5]. Bowel obstruction, chronic pelvic pain, infertility, relaparatomy and bowel or bladder injury during subsequent surgeries is long term complications of adhesions. Mechanism of adhesion is an inflammatory process during recovery process of traumatized peritoneum [2,6-9].

When the parietal and visceral peritoneum's are injured for any reason, mesothelial layer of base membrane will be connected with adjacent tissues and causes ischemia, inflammation, replacement of fibrin and collagen formation which causes adhesion [2-3-5-10-11].Although definitive etiology of pelvic's adhesions is unknown but there are some risk factors for example: manipulation of tissues during surgery, hypoxia and ischemia of tissues because of devascularization, infection such as bacterial peritonitis, foreign body, intra abdominal blood and free clots, pelvic inflammatory disease , endometriosis and etc [12-13]. Heparin is an anticoagulant drug which has widely useage for prevention and treatment of deep vein thrombosis and since the theoretical, fibrin formation and adhesion sequence will be stopped by heparin, it can be a suitable drug for abdominal surgery. some of the studies showed that heparin can be effective for prevention of adhesion after surgery and ultimately reduce the incidence and severity of adhesions [14-16]. Since the antibiotics can stop infectious process too, which cause inflammation and ultimately adhesion [17]. According to previous studies, antibiotics looks have an effective role in prevention of adhesion after surgery so it is better to use antibiotics against all of the gram positive, gram negative and anaerobics, so according to mentioned topics in this study we made a decision with use of heparin, clindamycin and cefepime identify the most effective combination for prevention of adhesion and evaluate separate and combination effects of them on adhesion in female rats.

MATERIAL AND METHOD

This was a case-control study that was performed on 8 groups of female rats with 10 rats in each group. The average weight of rats was 200-250 gr. They were kept in molecular-cellular laboratory with controlled environmental condition such as12 hours dark cycle،12 hours light cycle with 25ºc temperature. The animals were deprived of access to food 12 hours before surgery but access to water was free for them. During these times for prevention of eating feces, the animals were kept in small cages. At surgery time, first the animals were weighed to calculate the dose of anesthetic drug then they were anesthetized by 30 mg/kg intra peritoneal ketamin. We used inhaled ether if needed. After making sure the rat was unconscious, the skin of abdomen was shaved and disinfected by betadine then by scalpel No.15 the abdominal wall layers were opened with 3-4cm midline incision and other process was done in accordance with each groups , of course all of the surgeries were done by same person.

Group A (control): peritoneal cavity volume was considered 10 cc. In this group, at first the animals were under laparatomy then after abration and cutting over the peritoneal layer,2cc normal saline was poured into the peritoneal cavity and ultimately the abdominal wall was repaired with 4/0 nylon [18].

Group B (heparin): In this group of animals after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with 250 Iu heparin diluted with distilled water until 2cc and ultimately the abdominal wall was closed with 4/0 nylon [18].

Group C (cefepime): In this group of rats after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with 50mg/kg cefepime diluted with distilled water until 2cc and ultimately the abdominal wall was closed with 4/0 nylon [17].

Group D (clindamycin): In this group of rats after laparatomy,abration and cutting over the peritoneal layer, the peritoneal cavity was filled with 150 mg/kg clindamycin diluted with distilled water until 2cc and ultimately the abdominal wall was closed with 4/0 nylon[19].

Group E (heparin + cefepime): In this group of rats after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with heparin in accordance with group B and cefepime in accordance with group C and ultimately the abdominal wall was closed with 4/0 nylon [17,18].

Group F (heparin + clindamycin): In this group of rats after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with heparin in accordance with group B and clindamycin in accordance with group D and ultimately the abdominal wall was closed with 4/0 nylon [18,19].

Group G (cefepime + clindamycin): In this group of rats after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with cefepime in accordance with group C and clindamycin in accordance with group D and ultimately the abdominal wall was closed with 4/0 nylon [17,19].

Group H (cefepime + clindamycin + heparin): In this group of rats after laparatomy, abration and cutting over the peritoneal layer, the peritoneal cavity was filled with cefepime in accordance with group C and clindamycin in accordance with group D and heparin in accordance with group B ultimately the abdominal wall was closed with 4/0 nylon [17-19].

After the recovery of animals, they were kept in cages separately and the tail of them was marked with paint to identify groups. The animals were given food and water and for 2 weeks they were monitored. After that under general anesthesia at first the abdominal skin was shaved and disinfected then the abdomen was opened with U shape incision and the abdominal cavity were evaluated for the presence of

adhesion bands , All of these stages were done by another person. Severity of adhesion was determined on the basis of Majuzi table (adhesion grade from 1 to 5).[2-14-20]

Table 1: Majuzi adhesion severity scoring system

grade	Description
0	No adhesion
1	Low adhesion can be separated with surgical pence
2	Moderate adhesion can be separated with surgical pence, but require sharp dissection in less than 50% of the adhered segments
3	Severe adhesion require sharp dissection in more than 50% of the adhered segments
4	Serous membrane damage
5	Deeply affected serous membrane

Evaluation of pathology

Ultimately we prepared flattened samples from adhesion bands and target organs of all groups studied for example small bowel, colon, abdominal wall and finally they were kept in fixative(formaldehyd10%) and sent to the laboratory to do histologic evaluation [17].

RESULTS

In this study 80 female rats were divided in to 8 groups and they underwent laparatomy and multiple peritoneal scratches and use of drugs.

Table 2: Incidence and severity of adhesion and comparisons of the results within the groups

Groups	No	Severity of adhesion						Incidence of adhesion	The average of severity of adhesion
		0	1	2	3	4	5		
A(control)	10	2	5	3	0	0	0	8 / 10 = %80	**1.10**
B(heparin)	9	4	3	2	0	0	0	5 / 9 = %55.6	**0.78**
C(cefepime)	10	6	1	3	0	0	0	4 / 10 = %40	**0.70**
D(clindamycin)	10	4	5	1	0	0	0	6 / 10 = %60	**0.70**
E(heparin + cefepime)	10	5	5	0	0	0	0	5 / 10 = %50	**0.50**
F(heparin+clindamycin)	10	1	7	2	0	0	0	9 / 10 = %90	**1.10**
G(cefepime + clindamycin)	10	5	5	0	0	0	0	5 / 10 = %50	**0.50**
H (heparin + cefepime + clindamycin)	10	6	4	0	0	0	0	4 / 10 = %40	**0.40**

For statistical tests and analysis of results we used ANOVAs and Chi-square tests. One of the 80 female rats in group B(heparin)died 2 days after surgery because of bleeding. According to table No.2 the maximum adhesion were in groups A(control) and F(heparin + clindamycin) and the minimum adhesion were in groups C(cefepime) and H(heparin + cefepime + clindamycin) of course this difference was not significant (p-value>0.05). The average severity of adhesion in groups A (control) and F(heparin + clindamycin) were maximum and in group H (heparin + cefepime + clindamycin) was minimum. There is a significant difference between groups A (control) and H (heparin + cefepime + clindamycin) in comparison of average severity of adhesion (p-value=0.025) and there was significant difference between group F(heparin + clindamycin) and group H(heparin + cefepime + clindamycin) in comparison with other groups.

Comparing groups in pathological

In pathological we saw the minimum fibrosis, inflamation and vascular proliferation in group H(heparin + cefepime + clindamycin), in term of fibrosis there was significant difference between group A(control) with group G(cefepime + clindamycin) and group H(heparin + cefepime + clindamycin) ,respectively. (p-value=0.012 and p-value=0.024). In term of inflamation there was significant difference between group

A(control) and group E(heparin + cefepime) and group H(heparin + cefepime + clindamycin) (p-value=0.030). In term of vascular proliferation there is not significant difference.

DISCUSSION

Adhesion formation after surgery is one of the basic problems for every surgery. Evaluation of etiology and detection of prophylactic methods of adhesion formation can help to solve this problem. There are several studies with multiple drugs other systemic or local for this reason [21]. Heparin is an anticoagulant drug which theoretical stops fibrin formation and sequence of adhesion and it seems that it can be appropriate for abdominal surgery [14]. On the other hand since infectious process can induce inflammation and As a result adhesion formation, the theoretical antibiotics can reduce adhesion formation which prevent inflammation [17]. Studies showed that various materials such as antioxidants and antibiotics can reduce adhesion strength. The study Aram. S and et al vit C, the study Wollard. K and et al vit E and the study Najafian and et al sovery khoozestan can reduce adhesion formation [3,17,22,23].
Since the need for further studies in order to discover the best way to prevent intra abdominal adhesions and it seems that a combination of drugs can prevent infectious process, inflammation and fibrin formation is an effective method of preventing adhesion, in this study we evaluated the combination of heparin+ cefepime + clindamycin. The results of this study showed that use of this combination cause reduction of adhesion rather than other groups. Although it was not significant statistically there was a significant difference which could be related to small sample size. The intensity of adhesion was lowest in group H which was significant statistically between group A(control) and group H(cefepime + heparin + clindamycin)(p-value=0.025)
In the year 2010 the study Metin Kement and et al evaluated the effects of different doses of heparin (62.5 and 125 and 250 Iu) and seprafilm in Murine model. According to this study, maximum effect of the drugs in reducing adhesion was equal with 125 Iu and 250 Iu heparin which was comparable with seprafilm [14]. According to Durmus and et al study in 2011both heparin and pentoxifyllin could reduce adhesion and production of stress oxidative in rats but heparin is much more effective than pentoxifillyne in reducing adhesion in rat [24].The results of our study were different with Methin Kement study and Durmus study which expressed heparin can reduce adhesion formation, although in our study adhesion rate in heparin group was less than control group too but it is not significant statistically [14-24].According to Oncel M and et al study in 2001about evaluation of systemic effect of antibiotics in reducing adhesion, adhesion rate in cefepime group was less significantly. There were collagen bandles and inflammatory cells in control group but not only these bandles were much more less in cefepime group but also there were no inflammatory cell. According to this study the use of intra peritoneal cefepime during surgery reduces adhesion rate and strength [17]. In our study the lowest adhesion was in group C(cefepime) and group H(cefepime + heparin+ clindamycin) which coordinated the adhesion rate with the results of high study but it did not match the severity of adhesion. Until now there is no study to evaluate combination effect of heparin+ antibiotics we made a decsion to investigate these drugs effect alone and together in order for prevention of adhesion.
Results of this study showed that combination of heparin, cefepime and clindamycin can be effective in prevention of adhesion although there was no significant statistically but significant reduction was showed rather than other groups. In pathologic evaluation, minimal fibrosis, inflammation and vascular proliferation were seen in group H that except of vascular proliferation this difference was significant. Ultimately reduction of adhesion can be solved after surgeries problems of patients and can reduce rehospitalization and finally reduction of costs. It seems another study with more sample size is better to do for detection of effective way to prevent adhesion.

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