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The Efficacy of a Hyaluronate/Carboxymethylcellulose Membrane in Prevention of Postoperative Adhesion in a Rat Uterine Horn Model

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Kelekci, S., Yilmaz, B., Oguz, S., Zergeroglu, S., Inan, I. and Tokucoglu, S. *The Efficacy of a Hyaluronate/Carboxymethylcellulose Membrane in Prevention of Postoperative Adhesion in a Rat Uterine Horn Model*. Tohoku J. Exp. Med., 2004, **204** (3), 189-194 —— The current study was designed to investigate the efficacy of a hyaluronate/carboxymethylcellulose membrane for the prevention of post-operative adhesion in a rat uterine horn model. This is a prospective double-blinded controlled study. Nineteen injured uterine horns were treated with a hyaluronate/ carboxymethylcellulose membrane as a barrier and 19 injured uterine horns without any treatment served as a control group. Two weeks later, second look laparotomies were performed in order to assess the degree of the adhesion. Total adhesion scores, histopathological analysis of inflammation process and vascularization of adhesions were compared with control group. *P* values of 5% or less were considered statistically significant. Animals treated with a hyaluronate/carboxymethylcellulose membrane had significantly reduced post-surgical adhesion scores when compared with control group. In histopathological examination, there was less vascularization in the treatment group. The types and extent of inflammation were similar in two groups. Clinically and histopathologically, a hyaluronate/carboxymethylcellulose membrane is effective in reducing postoperative adhesion formation in the rat uterine horn model. ——— adhesion; barrier methods; hyaluronate/carboxymethylcellulose membrane; pelvic surgery

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Peritoneal adhesions develop in a majority of subjects during the post-operative period, with as much as 50 to 80% of gynecological surgical procedures resulting in the formation of adhesion (diZerega 1994; Monk et al. 1994). Adhesion formation after peritoneal surgery is a major cause of post-operative small bowel obstruction, female infertility, chronic pelvic pain, and difficult re-operative surgery (Ray et al. 1993; Strickler et al. 1994; Tulandi et al. 1998). Therefore, a method by which post-surgical adhesion formation could be reduced or prevented would be great benefit in reducing post-operative morbidity and mortality.

To reduce the formation of post-operative adhesions, many surgeons have developed a variety of surgical techniques and have used several agents. A mixture of chemically derivatized sodium hyaluronate and carboxy methylcellulose (HA/CMC) membrane has been tested as a surgical adjuvant in multiple animal models. This novel translucent bioresorbable membrane is a useful adjuvant in reducing the incidence, extent, and severity of abdominal post-operative adhesions (Becker et al. 1996; Beck 1997; Burns et al. 1997). In field of gynecology, there are few studies to test the efficacy of HA/CMC membrane (Farquhar et al. 2003). Furthermore, there is no prospective controlled trial on pathologic basis of pelvic surgery. So, we designed this study to improve our knowledge about efficacy of a HA/CMC membrane supported by pathologic findings. The aim of this study was to investigate the efficacy of a HA/CMC membrane in the prevention of post-operative adhesion in a rat uterine horn model both clinically and pathologically.

Materials and Methods

Animals

Twenty-six non-pregnant, female Wistar albino rats, weighing 190-230 g, were used as a model for evaluating post-operative adhesion formation. The animal investigation committee at Ankara Educating and Research Hospital approved this study. The animals were acclimated in the vivarum for a minimum of ten days before the studies were initiated and housed individually.

Experimental procedure

The rats were anesthetized by a single injection of ketamine hydrochloride (40 mg/kg; i.m. of Alfamin 10%, 100 mg/ml, Woerden, Holland) and xylanzine hydrochloride (2 mg/kg; i.m. of alfazyne 2%, 20 mg/ml, Woerden, Holland). The abdomen was shaved and prepared with a povidone-iodine scrub. A lower midline vertical incision, 3-4 cm in length, was made with no.10 scalpel blade. The uterine horns were brought through the incision. A 1.5 cm segment of each uterine horn beginning from the bifurcation was traumatized in seven spots on the antimesenteric surface using bipolar cautery. Apposite the parietal peritoneal surface of the sidewall of lower abdomen, the uterine horn was traumatized, and a 1.5 × 1.5 cm area between two main vascular branches of peritoneal surface was injured in the same fashion to create standard lesion with bipolar cautery in both sides of rats.

Before the main study, five rats were used to find the optimal dosage of bipolar cautery to creative standard lesion. The power of cautery was 20 watt and duration of application was one second for one spot. Following completion of the standard lesions, just before closure, a 2 × 2 cm HA/CMC membrane was used to prevent two surfaces being in contact on the left side of each rat. The right injured uterine horns served as an internal control group without any treatment.

The abdominal incision was closed in two layers. The musculoperitoneum and fascia were closed with simple interrupted suture of 4.0 polyglycolic acid. The skin was closed with simple interrupted sutures of 3.0 polyglycolic acid. The animals were allowed to recover completely and thereafter maintained separately with food and water ad libitum. Two rats died from anesthesia complication.

Second look laparotomies

Two weeks later, the animals were killed with cervical dislocation. A transverse subcostal

incision was made above the cephalad extent of lower midline laparotomy site and lesion site was inspected for the presence of adhesion. An observer blinded to group assignment performed adhesion scoring.

Adhesion scoring

The adhesion scoring system in this study is the same as the criteria in the study of Leach et al., 1998. Adhesions to uterine horn defect were scored as follows: 0 = no uterine adhesion; 1 = 1%-25% involvement; 2 = 26%-50%; 3 = 51%-75%; and 4 = 76%-100%. Adhesions were further characterized on gross examination according to severity as follows: 0 = no adhesion; 1 = filmy avascular; 2 = vascular or opaque; and 3 = cohesive attachment of uterine horn to ipsilateral abdominal sidewall. The degree of adhesion formation was evaluated with the following adhesion scores: 0 = no adhesion; 1 = the adhesion could be separated from tissue with gentle traction; 2 = the adhesion could be separated from tissue with moderate traction; and 3 = requiring sharp dissection. The sum of three parameters was used as the overall score for each group.

Histopathological examination

Adhesion-carrying tissues (uterine horn, bowel, bladder, and peritoneal surface) were excised en-bloc and fixed in formaldehyde solution. A pathologist blinded to the groups performed the histopathological examination of the specimens. Sections with a thickness of 5 μm were stained with hematoxylin and eosin and Mallory trichrome for light microscopy to evaluate the types and extents of inflammation process and vascularity. Furthermore, blood capillaries were identified by factor VIII immunolabeling. Types of inflammation were classified as acute, chronic and active-chronic according to cell types surrounding arterioles (polymorphonuclear leukocytes sign acute, plasmocytes sign chronic and polymorphonuclear leukocytes + plasmocytes + eosinophiles sign active-chronic inflammation process). Extent of inflammation was assumed as diffuse when inflammation process covered the whole area by light microscopy under high power (HPx10) magnification and assumed as focal when localized to a certain area. On the same areas, the number of capillaries was categorized as < 3, 3-10 and > 10 for subgrouping the vascularity.

Main outcome measures

Main outcome measures were total visual adhesion scores, the inflammation process, and vascularization of adhesions.

Statistics

Statistical evaluation was performed by means of SPSS 11.0 version (SPSS Inc., Chicago, IL, USA). Results are listed as mean ± S.D. Chi-square test was used for the comparison of histopathological findings and Wilcoxon's signed rank test was used to compare the treatment vs. control group's adhesion criteria. For determination of the relationship between body' weight, operation duration and adhesion criteria, Spearman rank correlation analysis was used. Differences were considered statistically significant when p values were less than 0.05.

Results

Our surgical procedures were well tolerated by animals. Total mortality rate was (2/26) 7.6%. All laparotomy sites were intact and none of the rats had an incisional hernia. Mean weight of rats was 208.7 ± 12.2 grams and mean operation duration was 18.3 ± 4.87 minutes. The uterine horn model was shown to be adhesiogenic, with 18 (94.7%) of 19 untreated uterine horns found to have adhesions. After treatment with a HA/CMC membrane, 4 (21%) of 19 uterine horns were totally free of adhesion. Furthermore, in the comparison made with regard to morphological appearance of the adhesions, it was determined that there was an evident difference between two groups. Adhesions in the treated group were localized to a certain area, were filmy and decreased in vascularity while the ones in the control group diffused to a broad area, were cohesive and in-

TABLE 1. *Comparison of HA/CMC and Control groups for reducing primary postoperative adhesions in the rat uterine horn model.*

Group (n)	Adhesion Score			
	Extent	Severity	Degree	Total
HA/CMC (n = 19)	1.26 ± 0.18	1.36 ± 0.22	1.52 ± 0.23	4.15 ± 0.58*
Control (n = 19)	2.63 ± 0.23	2.21 ± 0.18	2.36 ± 0.17	7.21 ± 0.52

Values are mean ± S.E.M.; n, number of uterine horns; HA/CMC, Hyaluronate/Carboxymethylcellulose; Control, untreated with a HA/CMC membrane.

* HA/CMC membrane vs. Control, $p = 0.003$, determined by Wilcoxon's signed rank test.

creased in vascularity.

The total adhesion scores and separate analysis of extent, severity, and density of adhesions in the HA/CMC membrane group are shown in Table 1. Separate analysis of the extent, severity, and density in the HA/CMC group showed significant differences for adhesion extent (p = 0.002), severity ($p = 0.016$) and degree ($p = 0.017$) when compared with controls.

There was lower number of vascularization in the HA/CMC membrane group than that of the control group ($p < 0.047$, Fig. 1) identified by factor VIII immunolabeling. As to the comparison of the inflammation process, there were no statistically significant differences between two groups in terms of the types and extents of the inflammation (Table 2). We did not observe any foreign body reaction in the tissues histo-pathologically.

When Spearman correlation analysis was made, we did not find any significant relationship between rat weights, operation duration and total visual adhesion scores (r1 = 0.221, P1 = 0.364, r2 = –0.033, P2 = 0.894).

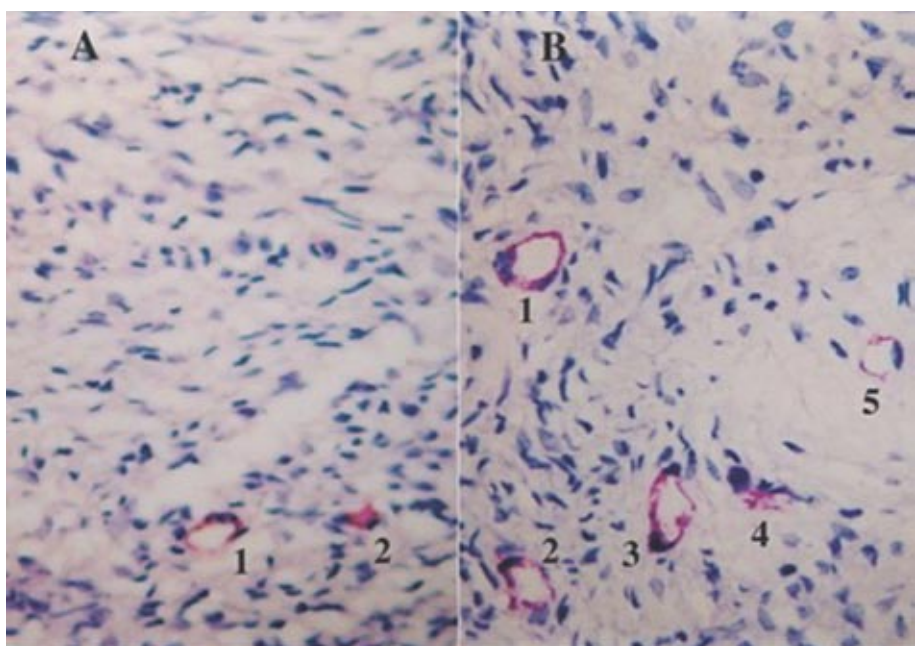

Fig. 1. Illustration of vascular content of adhesion-carrying tissues in both the treatment group (A) and control group (B). Numbers indicate blood capillaries identified by factor VIII immunolabeling.

TABLE 2. *Comparison of the histopathological findings between HA/CMC and Control groups.*

Parameter	HA/CMC group *n* (%)	Control group *n* (%)	*p* value
Type of inflammation			0.058
Acute	2 (10.5%)	0 (0%)	
Chronic	8 (42.1%)	12 (63.2%)	
Active-chronic	9 (47.4%)	7 (36.8%)	
Extent of inflammation			0.551
Focal	11 (57.9%)	8 (42.1%)	
Diffuse	8 (42.1%)	11 (57.9%)	
Vascularization (numbers of capillaries)			0.047*
< 3	5 (26.3%)	2 (10.5%)	
3-10	14 (73.7%)	10 (52.6%)	
> 10	0 (0%)	7 (36.8%)	

* Statistically significant.

DISCUSSION

This prospective double-blinded controlled study showed that the group treated with a HA/CMC membrane decreased postoperative adhesion formation. Moreover, adhesions were localized to a certain area, filmy in nature and decreased in vascularity in the HA/CMC membrane group compared with the control group. The HA/CMC membrane was significantly effective for reducing postoperative adhesion formation in rat uterine horn model. This has been proven both by morphological and histo-pathological examination.

Barrier adjuvants are used to prevent adhesions by decreasing the apposition of injured peritoneum until reperitonealization occurs. It turns into a hydrophilic gel approximately 24 hours after placement and provides a protective coating around traumatized tissues for up to 7-14 days during reepithelization (Raftery 1973). Therefore, we performed second-look laparotomies after 14 days of surgical procedures.

The ideal barrier should be nonreactive, bioabsorbable, and easy to use, and should persist during critical stages of healing. The HA/CMC membrane is extensively studied and has been approved for clinical use (diZerega 1994; Becker et al. 1996). Although there are few studies about it in the field of gynecology it is also effective in reducing the area of postoperative uterine adhesions after myomectomy (Diamond 1996). However, according to Cochrane review, there was no evidence of effectiveness of Seprafilm in preventing post-operative adhesion formation (Farquhar et al. 2003).

Leach et al. (1998) used HA/CMC gel, and achieved a reduction by 44% in the scores regarding extent, severity, and degree of adhesion formation. In our study, we observed a reduction by 42.3% in the total adhesion score and no adhesion in 21% of the HA/CMC membrane group. Our results are compatible with Leach's results. Reduction of tenacity of adhesion was prominent in our study. This character of adhesion has very close correlation with secondary surgical performance.

On the other hand, in our study, histo-pathologic investigation revealed that angiogenesis was significantly less in the HA/CMC group. This may be due to the mechanical barrier effect of a HA/CMC membrane, probably by preventing the deposition of fibrin gel on the extravascular space of the healing tissue as fibrin serves as a provi-

sional matrix that favors and supports the ingrowth of new blood vessels and other mesenchymal cells that generate mature, vascularized stroma (Dvorak et al. 1995).

Acute inflammation after peritoneal surgery is a major cause of postoperative adhesion formation. In acute inflammation, the peritoneal fluid shows increased concentration of the pro-inflammatory cytokines (Brauner et al. 1996). These cytokines interact with fibrinolytic system and play an important role in the adhesion formation process. Although in our study the difference was not statistically significant, acute inflammation process in the HA/CMC membrane group was less than in the control group.

Recently, the first reported case of a severe inflammatory reaction to HA/CMC membrane was presented (Klinger et al. 1999). This HA/CMC membrane induced foreign body reaction and induced inflammation. This previously unknown complication raises the issue of the safety of all bioresorbable membranes, which are increasingly used by surgeons. However, we did not observe any foreign body induced inflammatory reaction in our treatment group.

In conclusion, the HA/CMC membrane is effective for reducing postoperative adhesion formation in rat uterine horn model.

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