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The Effects of Hyaluronic Acid-Carboxymethylcellulose Membrane (GUARDIX-MB®) Barriers on Prevention of Post-operation Peritoneal Adhesions in Dogs

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Abstract : The aim of this study was to determine the effectiveness of hyaluronic acid-carboxymethylcellulose membrane (GUARDIX-MB®) barriers on prevention against post-operative peritoneal adhesions. In this study, fourteen mongrel dogs were divided into two experimental groups: 0.1 % hyaluronic acid (0.1HA) group and hyaluronic acid-carboxymethylcellulose membrane (HA-CMC) group. In order to induce adhesions, the anti-mesenteric serosa of the ileum was exteriorized and then abraded in a standard manner by scraping with a scalpel blade to create homogenous petechial hemorrhagic surface over a 1 × 1 cm area. Solution of 0.1HA were simply coated over the abraded tissues, 1.5 × 1.5 cm HA-CMC membrane was placed over the abraded tissues, allowed to spread across the intra-abdominal organs before closure of the abdomen. On day 1 before and day 1, 4, 7, 14, and 21 after operation, venous blood specimens were collected for measurement of fibrinogen and total WBC. The adhesions were blindly assessed 3 weeks later by using a computerized tensiometer. The fibrinogen and total WBC values of two groups showed no statistical significances. The mean tensile strength (gram force, gf) of formed adhesions day 21 after surgery was 88.1 ± 55.70 gf in the 0.1 % HA group and 24.8 ± 22.69 gf in the HA-CMC group. The tensile strength values of adhesion separation HA-CMC membrane group was significantly lower than the 0.1HA group ($p<0.05$). Therefore, we suggest that HA-CMC membrane reduce peritoneal adhesions may be applicable to preventing post-operative intraperitoneal adhesions in dogs.

Key Words **:** hyaluronic acid-carboxymethylcellulose membrane, peritoneal adhesions, tensile strength, dogs.

Introduction

Postoperative adhesions is an important and so far unsolved surgical problem (38,43). Adhesions were present in more than 94 % of patients who underwent major abdominal operations (4,5). Intra-abdominal adhesions are common cause of mechanical bowel obstructions (3,11,25), female infertility (6,17), and then they may be related to chronic abdominal pain (22,35). Adhesions are fibrinous or fibrous bands that form abnormal unions between two or more surfaces that are normally covered with the serosa (23).

Adhesion formation (10,20,21,30,31) begins with a fibrin matrix; cellular elements become prominent in the matrix at 1 to 3 days. Vascular granulation tissue containing macrophages, fibroblasts, and giant cells gradually replaces this matrix (19). After 4 days, most of the fibrin disappears, macrophages become the predominant leukocyte, and a larger number of fibroblasts and associated collagen are present. At day 5, small vascular channels containing endothelial cells are seen, and within the adhesion while collagen deposition and organization advance (15). During the second week, the relatively few cells present are predominantly fibroblasts. Mesothelium (13) often covers well-defined adhesions, which contain blood vessels and connective tissue fibers, including elastin (30).

To prevent or reduce the complication of intraperitoneal adhesions numerous studies have been done on animals (9). Various adjuvants have been tried to prevent postoperative adhesions, these are as follow: anti-inflammatory agents (NSAIDs, corticosteroids), antibiotics, rubricate (fluid) agents (normal saline, chlorohexidine, sodium carboxymethylcellose) (40), hyaluronic acid, dextran 70, and antioxidative agents (vitamin E). In the past decade, significant progress has been made with the development of adhesion barriers. These include Gore-Tex® membrane; oxidized, regenerated cellulose (Interceed®) (2,18); and hyaluronic acid-carboxymethylcellulose (HA-CMC) membrane (Seprafilm®; Genzyme, Somerville, NJ). Hyaluronic acid (HA) has been shown to prevent adhesion formation in both experimental models and human subjects (1).

HA-CMC membrane (GUARDIX-MB®) (7,26,39,41) is applied directly to specific sites of surgical trauma to provide a physical barrier that separates traumatized tissue from

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other tissues during normal healing. Composed of sodium hyaluronate and carboxymethylcellulose, HA-CMC membrane is slowly resorbed into the body, maintaining a barrier effect while the body's normal tissue repair takes place.

Accordingly the aim of this study was to evaluate the effectiveness of HA-CMC membrane barriers by comparing with HA capacity of which to prevent the formation of postoperative adhesions in a noninfectious environment is well known.

Materials and Methods

1. Experimental animals

Fourteen healthy adult mongrel dogs (weighing 4.9 ± 1.4 kg) were used in the study. They were vaccinated with DHPPL and dewormed with febantel (Drontal® Plus, Bayer Korea Ltd, Korea). Experiments were started after an initial adaption period for about ten days. The dogs were housed in each cage, and the food and water were fed *ad libitum*. All dogs were divided into two groups containing seven animals, respectively: 0.1 % hyaluronic acid-treated group (0.1HA), and hyaluronic acid-carboxymethylcellulose membrane (HA-CMC) group.

2. Preparation of materials

The HA-CMC membrane was kindly provided by BIORANE. Co., Ltd. The anti-adhesion membrane was prepared by lyophilizing HA-CMC solution and cross-linking properly with 1-ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDAC).

HA was used as a 0.1 % solution. 0.1 % HA solution was prepared by adding 22.5 ml of sterile water at 1 % HA (Sodium hyaluronate 25 mg/ 2.5 ml, HYAL®, Shinpoong Pharm Co, Korea) and then mixing with Vortex Genie 2, and filtering with syringe filter (0.45 Micron, 25 mm). All materials were stored at 4 °C until used.

3. Surgical Procedures

Feed was withheld from experimental dogs 12 hours prior to surgery. Anesthesia for surgery was similar for all groups. Each dog was administered atropine sulfate (Atropine®, Dai Han Pharm Co, Korea, 0.05 mg/kg) subcuticularly. Anesthesia was induced and maintained with intramuscularly tiletmine/zolazepam (Zoletil®, Virbac Co, Korea, 5 mg/kg). Dogs were positioned in dorsal recumbency and the entire abdomen was clipped. The abdomen of each dog was prepared with povidone-iodine solution and 70 % alcohol for aseptic surgery. Sterile surgical instruments and supplies were used in all surgical procedures. The surgical site was draped in a common manner. A 5 cm ventral midline incision was made through the skin, subcutaneous tissue, and peritoneum.

0.1HA Group: After the abdomen was opened, the ileum and ileocecal junction were examined and then exteriorized. Total five distinct surgical lesions were made in order to induce adhesions. The antimesenteric defects of the ileum approximately 5 cm from the ileocecal junction were created by light scraping about 1 × 1 cm areas with a sterile No. 10 scalpel blade to promote petechial bleeding, and allowed to air dry for 10 minutes. The total abrasions were 5 places at intervals of 5 cm. In this group, solution of 0.1HA were simply coated over the abraded tissues (3 ml/abraded area). And then the small bowel was returned to its original location. Before closure of the abdomen, liquid product was allowed to spread across the intraperitoneal organs (3 ml/kg) through a catheter using a syringe. The catheter was removed, and abdominal wall was closed using a simple interrupted pattern with 2-0 polyglactin 910 (Vicryl®, Johnson&Johnson Medical Korea Ltd, Korea) and the skin was closed by a simple interrupted pattern with 3-0 nylon (BLUE NYLON®, Ailee Co, Ltd).

HA-CMC Group: Antimesenteric defects to induce adhesions were created at five locations using a similar method described 0.1HA Group. In this group, 1.5 × 1.5 cm HA-CMC membrane was placed over the abraded tissues. The abdominal cavity was closed using with same method used in 0.1HA group.

One surgeon carried out all the operations and was blind to the randomization while he was performing the abrasions.

4. Postoperative Evaluations

The experimental animals were monitored daily for signs of abdominal pain, drainage, feed consumption or defecation. Enrofloxacin (Baytril, Bayer Korea Ltd, Korea, 5 mg/kg) was administered subcuticularly to reduce the risk of postoperative infection for 3 days.

Fibrinogen and WBC values : On day -1 (day 1 before operation), 1, 4, 7, 14, and 21 after operation, venous blood specimens were collected from all experimental animals for measurement of fibrinogen and total WBC.

Adhesion Evaluation : Three weeks later, all the animals were sacrificed and the intra-abdominal cavity was inspected through ventral midline incision. And then a postmortem examination was conducted immediately. Adhesions were identified, and the adhesion site was excised to test. The tensile strength of the adhesion site was evaluated with tensiometer (H500DM®, Hounsfield Co, UK) (Fig 1). Both adhesive tissue ends were secured tightly in a clamp so as not to slip from the clamp during tensile test. The clamp was advanced at the rate of 0.7 cm/min. During the operation of tensiometer, breaking strength of specimens was measured. Tests were performed at the room temperature of 20 °C, and specimens were moistened with a very fine mist of normal saline while clamped.

5. Statistical analysis

Values are expressed as mean ± SD. Analysis of differences between treated groups was performed using analysis of variance followed by Student's t test. Differences in the separation strength of two groups at $P<0.05$ were considered statistically significant.

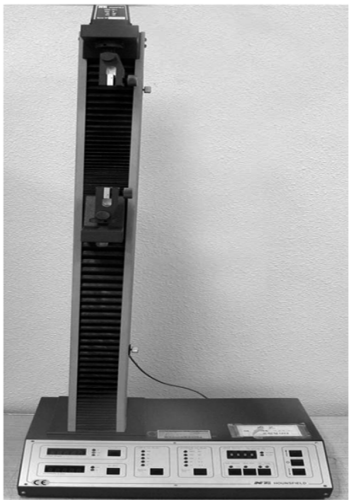

Fig 1. The tensiometer used in the measurement of the force required to separate adhesions.

Results

The second laparotomy was performed in all experimental groups at 3 weeks after surgery. All animals had insignificant postoperative signs during the experimental periods.

1. Laboratory Values

Fibrinogen: The fibrinogen concentration was normal on day -1 in all dogs, On day 1, values of fibrinogen was increased higher than those on day -1. The concentration of fibrinogen of 0.1HA group was decreased gradually from day 7, and recovered to normal ranges on day 14. The concentration of fibrinogen in HA-CMC group was decreased gradually from day 4, and recovered to normal ranges on day 21 (Fig 2). There was no statistical significance between the two groups.

WBC: The total WBC was normal on day -1 in all dogs, On day 1, they were increased higher than those on day -1. Total WBC numbers decreased gradually from day 4, and recovered from normal ranges on day 14 (Fig 3). There was no significant difference between the two groups.

2. Tensile Strength

All of the animals in 0.1HA group were admitted for occurrences of adhesions. Adhesions were occurred among five animals in HA-CMC group except two animals (Table 1).

The mean adhesion separation strength (gram force, gf) was 88.1 ± 55.70 gf in 0.1HA group, and 24.8 ± 22.69 gf in HA-CMC group. Values of adhesion formation in HA-CMC group was significantly reduced, compared with those in 0.1HA group ($P<0.05$) (Table 2).

Adhesions were identified in total sites; serosa to serosa (18 of 70, 25.7 %), serosa to mesentery (27 of 70, 38.6 %), serosa to omentum (5 of 70, 7.1 %), serosa to parietal peritoneum (2 of 70, 2.9 %). Total number of adhesions was 29 of 35 in 0.1HA group, and 23 of 35 in HA-CMC group (Table 3).

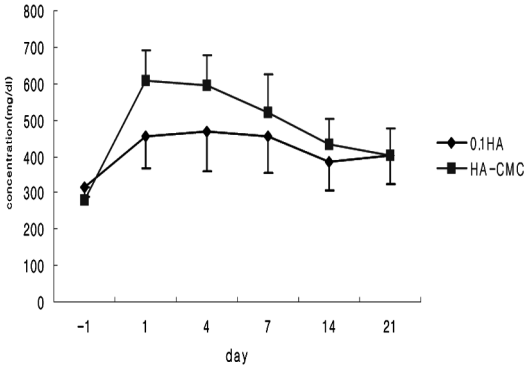

Fig 2. Changes of fibrinogen concentration of blood serum over time in dogs intraperitoneal adhesion was induced. 0.1HA : 0.1 % hyaluronic acid-treated group HA-CMC : hyaluronic acid-carboxymethylcellulose membrane-treated group. Mean ± SD.

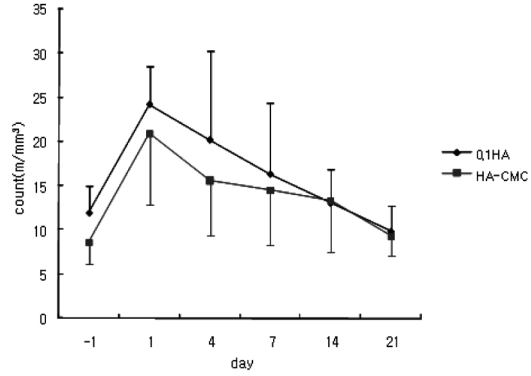

Fig 3. Changes of total WBC count in dogs intraperitoneal adhesion was induced. Mean ± SD.

Table 1. Adhesion incidences in each dog of groups on day 21 after operation (no. of adhesion sites

Group	No. of animal							Total
	1	2	3	4	5	6	7	
0.1HA	3	3	6	3	4	7	3	29/35
HA-CMC	4	7	0	2	7	3	0	23/35

The mean strength (gram force, gf) of the adhesion separation in each sites ; serosa to serosa (121.8 ± 107.65 gf), serosa to mesentery (61.0 ± 31.64 gf), serosa to omentum (16.5 ± 2.12 gf), serosa to parietal peritoneum (306 gf) in 0.1HA group, and serosa to serosa (56.3 ± 39.57 gf), serosa to mesentery (35.8 ± 19.76 gf), serosa to omentum (7.3 ± 4.04 gf), serosa to parietal peritoneum (56 gf) in HA-CMC group (Fig 4).

Gross appearance of intraperitoneal adhesion in 0.1HA group was dense and broad, but that in HA-CMC group was

Table 2. The mean strength values of the adhesion separation in each dog of groups

Group	No. of animal							Average (mean ± SD)
	1	2	3	4	5	6	7	
0.1HA	60.6 ± 14.97	201.3 ± 153.08	102.1 ± 101.95	327.6 ± 14.50	454.5 ± 50.73	80.4 ± 75.67	90 ± 16.70	88.1 ± 55.70
HA-CMC	26.2 ± 22.85	732.7 ± 13.11	0	224.5 ± 2.12	67.1 ± 38.42	23.3 ± 28.57	0	24.8 ± 22.69*

* $p<0.05$ compare with the 0.1HA

Table 3. Postoperative locations of adhesions in dogs 21 after operation (no. of adhesion sites)

	Location				
	Total	S-S	S-M	S-O	S-PP
0.1HA	29	10	16	2	1
HA-CMC	23	8	11	3	1

Locations of adhesions : S-S = serosa-serosa ; S-M = serosa-mesentery ; S-O = serosa-omentum ; S-PP = serosa-parietal peritoneum.

filmy and thin (Fig 5).

Discussion

Injury or inflammation of a serosal surface generally initiates adhesion formation processes (8). Destroyed peritoneum fails to absorb fibrinogen-rich fluid exudate. In addition activation of inflammatory cells (42), secretion of inflammatory cytokines, and activation of the complement-coagulation cascades creates thrombin, which converts fibrinogen to fibrin in the exudate (24). Fibrin deposition not degraded within the first days of injury results in the progression of a fibrinous adhesion into a fibrous form with the movement and propagation of fibroblasts into the fibrin matrix. If degradation occurs, mesothelium (13) repairs its defect without adhesion formation (10,16,20,21,30,31). Small bowel is the most frequently observed location of adhesion formation because it occupies most of the abdomen and in located in the middle of the peritoneal cavity(14). For these reasons, it is very important to identify an anti-adhesive

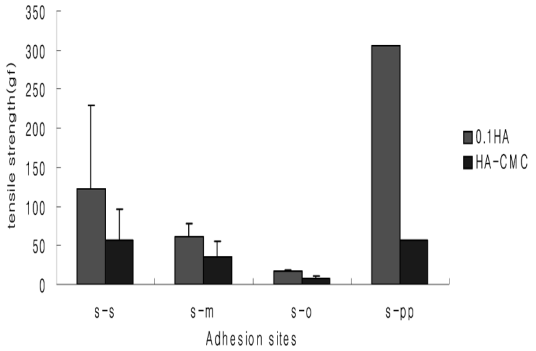

Fig 4. The strength values of the adhesion separation in each sites.

product to prevent adhesions, especially between small bowel segments and between small bowel and other organs. Therefore, we abraded illeum to induce adhesion in our experiment.

Numerous agents have been used to reduce postoperative adhesions. No agent has yet proved effective against the formation of intra-abdominal abscesses and adhesions in bacterial peritonitis in patients. A promising new concept may be the application of intra-abdominal agents (12) containing the polysaccharide hyaluronan (36), which hae been shown to prevent adhesions in experimental and clinical studies (32). HA, a naturally occurring glycosaminoglycan, forms a highly viscous solution coating the serosal surfaces. Many studies showed HA decreases inflammation (34), interferes with

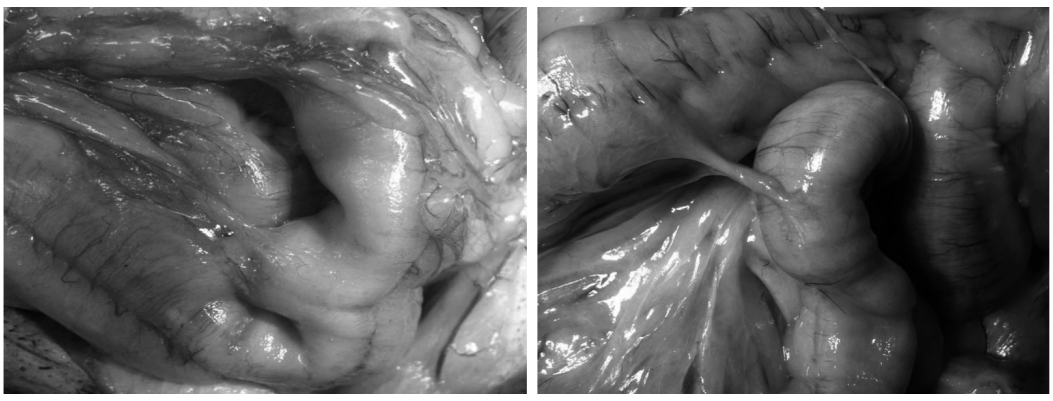

Fig 5. Gross appearance of adhesiolysis in dogs intraperitoneal adhesions was induced. 0.1HA (left) group and HA-CMC (right) group.

fibrin formation, prevents adhesion (29), and stimulates fibrinolytic and TNF-α (27) response. Preventing the formation of postoperative adhesions among these reactions (33) is believed viscous polysaccharides solutions reduce serosal trauma by coating the peritoneal surfaces which become damaged during abdominal surgery.

Application of a mechanical barrier between the injured peritoneum is a method to prevent adhesion formation. HA-CMC membrane is such a barrier (28). It is a biologically resorbable membrane, which composed of HA absorbed CMC film. The ideal barrier should be non-reactive, absorbable and easy to use, and it should remain in the lesion site during critical stages of healing. The safety and efficacy of this membrane in preventing postoperative adhesion has been shown in clinical and experimental studies (39). Therefore, we supposed that the HA-CMC membrane functions as a physical barrier by separating the serosal surfaces may be beneficial in the treatment of intraperitoneal adhesions related to inflammation and fibrin.

Bleeding is likely to cause a massive extravasation of fibrinogen into the peritoneal cavity. Adhesion formation begins with fibrin deposition within an inflammatory exudate formed over an injured serosal layer (37). Our result showed that the fibrinogen concentration of HA-CMC treated group were higher than those of 0.1HA treated group on day 1 and 4, but were decreased gradually from day 4, and recovered to normal ranges on day 21. We have no idea about the reason the fibrinogen concentration of HA-CMC treated group was higher. Further studies is warranted on the relation of HA-CMC membrane and fibriongen concentration.

The value of total WBC was normal on day -1 in all dogs. On day 1 after operation, total WBC were increased higher than those on day -1. Total WBC concentraton decreased gradually from day 4, and recovered to normal ranges on day 14. In this study, we suppose that total WBC count increase is resulted from inflammation after operation and the change of total WBC count could be caused by the inflammatory reaction rather than change in the adhesion formation.

In present study, we performed by using a HA-CMC membrane and 0.1 % HA solution. All of the animals in 0.1HA group and five animals in HA-CMC group were admitted for occurrences of adhesions. Values of adhesion formation in HA-CMC group was statistically significant reductions in compared with those in 0.1HA group ($P<0.05$). In this results, we can consider that HA-CMC membrane reduced the incidence and severity of adhesion because of the physical properties of the membrane; Reduce fibrin deposits by limiting the inflammatory response, facilitate the degradation of fibrin with fibrinolytic stimulators and separate surfaces during the time that the fibrin remains sticky. The results are in agreement with similar studies (39). Almost all studies related to HA-CMC membrane were about the physical or barrier effects of the material. HA-CMC membrane is a temporary proven to reduce the incidence, extent, and severity of adhesions in patients undergoing abdominal or pelvic laparotomy. It was designed to prevent adhesions by separating traumatized tissue surfaces (44). When applied to traumatized tissues, HA-CMC membrane hydrates and becomes a gel and remains in place during the critical 7-day healing period. One limitation of HA-CMC membrane is its handing characteristics. It is somewhat brittle and sometimes difficult to apply. Theoretically, dislocation is possible after application, and this may interfere with the membrane's anti-adhesions effect.

Therefore we suggest that the HA-CMC membrane was effective on preventing the formation of postoperative small intestinal adhesions in the dog. Further studies about histological analysis are needed to find inflammatory reaction and fibrotic response in adhesive sites.

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