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 0.1HA 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
The Effect of Guardis-MB® on Prevention of Post-Operative Peritoneal Adhesions in Dogs

1. Experimental animals

Fourteen healthy adult mongrel dogs (weighing 4.9 ± 1.4 kg) were used in the study. They were vaccinated with DHPPiL and dewormed with febantel (Drontal® Plus, Bayer Korea Ltd, Korea). Experiments were started after an initial adaptation 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 BIO-RANE, 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 tiletamine/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 a very fine mist of normal saline while 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.