The Effect of Adhesion Barriers in Preventing Pericardial Adhesion Depending on the Type of Barrier: Solution Type versus Film Type

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Background: Pericardial adhesion is a significant challenge for re-sternotomy. An adhesion barrier made with hyaluronic acid and carboxymethylcellulose (HA-CMC) has been used. The solution type and film type of HA-CMC barrier was commercially introduced. While the solution type is easy to apply but possible to stream down the wound sites, the film type showed fixation on the wound sites but difficulty in handling. This study was designed to investigate the effect of adhesion barriers according to the type of barriers.

Methods: Twelve mongrel dogs were classified into two group; the S group (n=6) which received the solution type of barrier, and the F group (n=6) which was received the film type. After making an abrasion wound on left ventricular wall, the wound was covered with a solution or film type of barrier. After 4 weeks, adhesion severity (Grade 0-5) and adhesion strength (grade 0-4) were measured. The number of white blood cells (WBC), rate of erythrocyte sedimentation (ESR), and the amount of serum C-reactive protein (CRP) were measured for the estimation of systemic inflammation at 1 week and 4 weeks postoperatively. Microscopic examination was also performed. Results: Adhesion severities of the S and F groups were both scored as 4. The adhesion strengths of the S group and F group were 4 and 3, respectively. There were no significant differences between groups when WBC count, CRP, and ESR were compared. Degrees of fibrosis, regeneration of the mesothelial cell, inflammation, and foreign body reaction were not also significantly different between the groups. Conclusion: Although the concern that solution type of barrier might be decreased the anti-adhesion effect because of the possibility of streaming down the wound sites, the anti-adhesion effect was not significantly different between groups. These results may help surgeons decide the appropriate type of adhesion barrier to use.

Key words: pericardium, animal model, biomaterials

Introduction

Postoperative pericardial adhesion formation is a significant cause of morbidity and mortality associated with cardiac surgery. There is a reported 2 to 6% incidence of catastrophic hemorrhage during re-sternotomy; meticulous dissection carried out to free the heart and great vessels, and to avoid injury to vascular structures prolongs the operation.¹

Primary closure of the pericardium is therefore recommended to reduce the formation of adhesions between the heart and chest wall.² However, autologous pericardium cannot be used in some patients for various reasons. Many experimental and clinical attempts have been made to solve this problem by using different types of pericardial substitutes. Of the available synthetic pericardial substitutes, those made from expanded polytetrafluoroethylene (e-PTFE) are known to be superior to others made from different materials.³ However, clinical use of e-PTFE has been limited by concerns that this material is non-absorbable, so permanence of the sheet could induce an extensive inflammatory reaction resulting in formation of a fibrous capsule. This poses a potential problem for pediatric patients as the heart grows, and leaving a foreign body in place predisposes patients to infection over time.⁴

Due to these concerns, a variety of approaches to make absorbable adhesion barriers have been employed. Hyaluronic acid-carboxymethylcellulose (HA-CMC) is one of the absorbable materials which were used as an adhesion barrier. HA is a ubiquitous high molecular weight substance normally contained in the synovial fluid, the vitreous humor, and the extracellular matrix. It is a glycosaminoglycan made of repeating disaccharide units of glucuronic acid and N-acetylglucosamine, which acts as a barrier substance and lowers the severity and intensity of adhesion resulting from the inhibition of fibrin formation by coating the exposed area and saturating the CD44 receptor of peritoneal mesothelium.⁵⁶ On the other hand, CMC is a relatively low molecular weight and water-soluble substance that is generated by the chemical modification of cellulose. Because the human body does not have enzymes to degrade it, it is not immediately absorbed and remains in the surface of tissue during mucosal healing, thus acting efficiently.

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as a physical barrier. HA has limitations as a physical barrier because it is degraded easily by hyaluronidase causing its half-life to be only 1-3 days. As a means to overcome this limitation, it is used in combination with CMC, because CMC functions as a chemical bridge of HA and acts as a conglutinant as well as coating substance. Hence, the use of combination of these two is more effective than its single use. HA-CMC was evaluated in several animal experiments, and different groups demonstrated its efficacy in clinical studies of gynecological and visceral surgery.

Different types of HA-CMC adhesion barriers have been developed, such as a film type and solution type, according to their application purposes. The film type (Separfilm® Adhesion Barrier, Genzyme Biosurgery, Cambridge, U.S.A.) is biocompatible, non-immunogenic, sticks to tissue surfaces, covers tissue surfaces by forming a viscous gelatinous gel, and is fully absorbable within 28 days. However, it is brittle, sticks to surgical gloves, and requires accurate placement at the site of injury to be effective. The solution type adhesion barriers (Guardix-sol., HamiMedicare, Seoul, South Korea) can easily spread over and widely coats the surface of tissue. However, one concern about the solution type is that it could be washed away by gravity. If the solution flowed out of the wound sites, the effect of the adhesion barrier could be decreased.

Although there have been many studies about the ability of adhesion barriers to prevent adhesion after sternotomy, the application of solution type of HA-CMC barrier on sternotomy has rarely been studied. Clinically, choosing the proper type of adhesion barrier could be helpful for decreasing the morbidity and mortality when patients need re-sternotomy.

In this work, we evaluated the efficacy and safety of different types of adhesion barriers, such as film and solution consisting of HA-CMC, in a dog model.

**Material and Methods**

**Animal model**

All experimental animals were cared for and treated according to the "Guide for the Care and Use of Laboratory Animals" issued by the Korea University School of Medicine, Seoul, South Korea. Twelve mongrel dogs (20-30 kg) were randomly assigned into two groups for in vivo evaluations of two different types of adhesion barriers such as solution (S group, n=6) and film (F group, n=6).

**In vivo experimental method**

An intravenous line was established with a 20 gauge catheter, and electrocardiographic monitoring was done. After injection of thiopental sodium (5-10 mg/kg), endotracheal intubation was performed. Maintenance of general anesthesia was achieved using 1-3% isoflurane administered via an endotracheal tube with a gas mixture of N<sub>2</sub>O/O<sub>2</sub> (1:1) at a flow rate of 2 L/min of each reagent. Mechanical ventilation was maintained with a tidal volume of 10-15 mL/kg and a respiratory rate of 30-35 breaths/min. Vecuronium bromide (4 mg) was added for the prevention of spontaneous breathing. Surgical procedures were performed by a single surgeon to reduce possible bias. Under sterile conditions, a left lateral thoracotomy incision was made through the fifth intercostal space. After making an opening in the pericardium longitudinally about 5 cm in length, the left ventricle was exposed. A small amount of pericardial fluid was collected for bacterial culture. A focal square area, 3 × 3 cm<sup>2</sup>, was marked with four sutures of 6-0 polypropylene sutures (Prolene, Ethon Inc, Somerville, New Jersey) on the left ventricle.

To promote adhesion formation, the marked epicardium of the anterior left ventricle was abraded 100 times with dry gauze. 1.5 mL solution type adhesion barriers (Guardix-sol., HamiMedicare, Seoul, South Korea) were applied onto the abrasion site in the S group. The abrasion sites were also covered with 3 × 3 cm<sup>2</sup> square adhesion barriers (Separfilm® Adhesion Barrier, Genzyme Biosurgery, Cambridge, U.S.A.) in the F group. At the end of the procedure, the pericardium was closed using five stitches of 6-0 polypropylene sutures, and the wound was closed layer by layer. At 1 week and 4 weeks after operation, blood samples were taken for measuring the number of white blood cells (WBC), the rate of erythrocyte sedimentation (ESR), and the amount of serum C-reactive protein (CRP). After 4 weeks following the initial operation, general anesthesia was performed with the same manner. Median sternotomy was done for exposing the pericardium. After making a small incision in the pericardium, pericardial effusion was collected again for bacterial culturing. The en bloc resection of the heart including the entire pericardium was done after euthanasia. The investigator who participated in scoring the adhesions had no prior knowledge as to which group the dogs belonged.

**Bacterial culturing**

After collection of pericardial fluid at the operation day and at 4 weeks after operation, bacterial culturing was done to evaluate the possibility of infection.

**Assessment of the degrees of tissue adhesions**

Vlahos’s scoring system was used for evaluating degrees of adhesion of tissues between the epicardium and pericardium. Adhesion severity was classified into six groups from 0 to 5 depending on the extent of tissue adhesion as follows: 0 = no adhesions, 1 = one thin filmy adhesion, 2 = two or more thin filmy adhesion, 3 = thick adhesion with focal point, 4 = thick adhesion with planar attachment and 5 = very thick vascularized adhesion. Adhesion strength was also classified into four groups from 1 to 4 as follows: 1 = adhesion was filmy and easily torn with very light pressure, 2 = adhesion was substantial and needed moderate pressure to tear, 3 = adhesion was heavy...
and required significant pressure to rupture, and 4 = adhesion
was very heavy and difficult to rupture.

Histological study
Specimens were taken from two points in the experimental
dogs. The first point was on the center of the square that was
made during scrubbing the epicardium. The second point was
at 3 cm apart from the center of the square (0 cm). The speci-
mens were stained using routine methods with hematoxylin &
esolin Y (H&E) for the whole tissue and with Masson trichrome
(MT) dye for collagen. Microscopic examinations were per-
formed by a single pathologist to reduce possible bias.

Assessments of tissue formation, inflammation and for-
eign body reaction
Microscopic evaluations were performed to measure the
extent of fibrosis, degree of regeneration of mesothelial cells
(neomesothelium), and degree of inflammation and foreign
body reaction at both the 0 cm and 3 cm locations. The degree
of fibrous formation was scored by employing the methods sug-
gested by Lu and colleagues. The extent of fibrosis was clas-
sified into four degrees of thickness (0 to 3) of both collagen
formation and deposition of fibrin depending on the thickness
of the regenerated fibrosis on the injured sites 4 weeks after
formation and deposition of fibrin depending on the thickness
of both categories such as negative and positive.

Statistical analysis
Categorical variables were listed as the median, and all the
continuous variables were listed as the mean ± standard devia-
tion. The data analysis was performed with SPSS (SPSS version
12 for Windows, SPSS Inc., Chicago, IL, USA). Mann-Whitney
U test was done for analysis of continuous variables. Pearson’s
chi-square test was done to compare the result of inflammation
and foreign body reactions between groups. Other categorical
variables of the two groups were compared with Score test for
trend. A p-value (< 0.05) was considered statistically significant
for all tests.

Results
Evaluation of pericardial fluid and bacterial cultures
In both groups, small amount of pericardial fluid was noticed
in the pericardial cavity at week 4; however, the color and
nature of the fluid was found clear by gross examination. Bac-
terial cultures of pericardial fluid at an operation day and week
4 post-operation were verified as negative except one. Enterob-
acter cloacae was detected in the one sample from the mem-
brane film group at 4 weeks after operation, however it was
considered as contaminated because enterobacter normally
grows in intestines.

Evaluation of systemic inflammation
The number of WBC, ESR, and the amount of CRP were
measured 1 week and 4 weeks after operation to evaluate the
degrees of systemic inflammation that may have been induced
by the adhesion barriers. At 1 week, the WBC count in the S
group and F group was 13.25 ± 4.27 × 10^3/µL and 13.69 ± 4.30
× 10^3/µL, respectively. The difference between the groups was
not significantly different (p = 0.94). The ESR of the S group
and the F group on 1 week after operation was 3.83 ± 2.86
mm/hr and 2.0 ± 0.0 mm/hr, respectively. The difference be-
tween groups was not significant (p = 0.59). The CRP of the S
group and the F group on 1 week after operation was 0.42 ±
0.22 mg/L and 0.17 ± 0.12 mg/L, and there was no significant
difference between the two values (p = 0.13).

At 4 week after operation, WBC counts of the S group and the
F group were 11.80 ± 3.53 × 10^3/µL, and 11.23 ± 3.45 × 10^3
/µL, respectively. These WBC counts were not statistically signifi-
cant different (p = 1). The ESR of the S group and the F group
was 4.67 ± 4.54 mm/hr and 2.0 ± 0.0 mm/hr, respectively, and
there was no significant difference (p = 0.39). The CRP of the S
group and the F group was 0.14 ± 0.18 mg/L and 0.16 ± 0.20
mg/L, and there was no significant difference (p = 1).

Evaluation of the degree of adhesion
The degree of adhesion severity and strength was evaluated
at 4 weeks after operation (Figure 1). There was no adhesion
formation in one sample of the S group and two samples of
the F group at week 4. However, the median degree of adhe-
sion severity for both groups was 4 (p = 0.75; Figure 2A). The
degrees of adhesion strength were measured as 4 for the S
group, and 3 for the F group. There was no significant differ-
ce (p = 0.40; Figure 2B). Adhesion size was 11.5 ± 7.79 cm
in the S group and 9.96 ± 9.32 cm² in the F group, and there
was no statistically significant difference between the two val-
ues (p = 0.96).

Histological study
Inflammation and foreign body reaction
Inflammatory reactions under the pericardial tissue were
observed in some specimens (Figure 2). Three cases in the S
group showed an inflammatory reaction at the 0 cm location,
and 4 cases in the F group showed an inflammatory reaction
the same site, but there was no significant difference (p =
0.56). At the 3 cm location, four cases in the S group and 5
cases in the F group showed an inflammatory reaction, but these were not significantly different (p=0.51).

Foreign body reaction was observed in one case in the S group and 1 case in the F group at the 0 cm location, but there was no significant difference (p = 1.0). Five cases in the S group and 2 cases in the F group showed a foreign body reaction at the 3 cm location, but there was no significant difference (p = 0.08).

Formation of pericardial fibrosis
Fibrosis of pericardial tissue was observed with MT staining.
(Figure 4). The median degree of fibrosis observed in the S group was lower than that of the F group at 0 cm, but there was no significant difference (1.5 versus 2, \( p = 0.52 \)). At the 3 cm location, the median values of fibrosis were 0.5 in the S group and 2 in the F group, but were not significantly different (\( p = 0.14 \)).

Regeneration of mesothelium
Some differences in the median grade of mesothelium regeneration and lining of a monolayer of mesothelial cells were demonstrated depending on the location, i.e. the 0 cm and 3 cm locations (Figure 5A, B). The median grades of mesothelium regeneration at the 0 cm location were 0 for both the S group and F group. At the 3 cm location, the median grades of mesothelium regeneration in the S group and in the F group were 1 and 2, respectively. The grade of mesothelium regeneration at either biopsy point did not differ significantly between the groups (\( p \) value of the 0 cm location = 0.72, \( p \) value of the 3 cm location = 0.32).

**Discussion**

We evaluated the efficacy for preventing pericardial adhesion of two different types of adhesion barriers in a dog model. The median score of adhesion severity was not significantly different between the two groups. Even though the S group displayed a substantially higher median value of adhesion size and strength than the F group, there was no significant difference between two groups. From this result, we thought that the effect of the solution type adhesion barrier is similar with the film type even though the solution type could have a chance to spill by gravity and could not stay on the abrasion site because of flowing.

The two groups showed similar biocompatibilities when they were tested for WBC count, ESR and CRP which indicates systemic inflammation. Also, the inflammation and foreign body reactions which wereshowed by microscopic examination were not significantly different between the groups. The results of the pericardial fluid cultures showed the chance of infection is not different according to type of barrier. From these results, we could assume that the type of adhesion barrier did not affect biocompatibilities.

Following injury, the native pericardium responds with a generalized inflammatory response with subsequent mesothelial cell desquamation, fibrin deposition over denuded serosal surfaces, and eventual fibrosis between opposing parietal and visceral surfaces.\(^{19,20,21}\) Pericardial surfaces are known to possess inherent fibrinolytic activity, which is reduced after mesothelial damage has occurred.\(^{20,21}\) Significant fibrinolytic activity returns to the pericardial cavity by the sixth post-injury day, coinciding with collagen deposition.\(^{19}\) Thus, the mesothelial cell has important role in preventing pericardial adhesion. In this study, we compared the degrees of mesothelial cell regeneration between two groups which were not found to be statistically significantly different.

The fibrosis formation was considered evidence of unwanted malicious tissue regeneration in this study because it induces tissue adhesion between the pericardium and epicardium during wound healing. In this study, the fibrosis formation in the two groups was not statistically significantly different. Therefore, the efficacies of the two adhesion barriers types were not different as assessed by microscopic examination.

There were a number of limitations in this study. First, the study included a small number of animals. Additionally, the results of the experimental findings cannot be directly transferred to the clinical setting. Since the epicardial damage in this study was limited, bleeding was minimal and cardiopulmonary bypass (CPB) was not performed. CBP itself could make damages on the mesothelial cells,\(^{22}\) so performing of CPB could
induce more pericardial adhesion.

Besides HA-CMC based barrier which we used in this study, several synthetic barriers with different characteristics are commercially available. Oxidised regenerated cellulose was the first tested synthetic mechanical barrier to cover traumatised peritoneum in the pelvis. Interceed (Johnson & Johnson, Cincinnati, USA) can be cut as necessary, requires no suturing and is absorbable. A new product is the fibrin sheet (Tacho Comb, Tokyo, Japan) which is a sheet-type fibrin sealant with a solid layer of human fibrinogen, thrombin and aprotinin coating the active surface of equine collagen stabilized with riboflavin. It is applied over raw tissue surfaces at the end of an operation. But the efficacy of these materials were evaluated based on visceral or gynecological surgeries not cardiac surgeries. In future studies, the efficacy of HA-CMC based barrier should be compared with other material with model of cardiac surgeries.

In conclusion, we evaluated the efficacy of two types of HA-CMC barrier, solution and film, in a dog model. There were no statistically significant differences in the degrees of adhesion for efficacy. The safety of barriers which was shown by inflammatory reactions observed with microscopy, and systemic inflammatory reactions seen when using either type of barrier as indicated by blood tests were not significantly different. The solution type of adhesion barrier was as effective as the film type.

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References