J Vet Clin 26(6) : 547-555 (2009)

Journal of Veterinary Clinics
www.ksvc.or.kr

Dose Related Effects of Poloxamer/Sodium Alginate Mixture in Prevention of Postoperative Adhesion Formation in Dogs

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(Accepted : December 09, 2009)

Abstract : This study was performed to determine the minimum dose of Poloxamer/Sodium alginate (PX/SA) mixture on preventing intraperitoneal adhesions to evaluate organ toxicity. Twenty five healthy adult mongrel dogs (weighing 4.68 ± 1.67 kg) were divided into five experimental groups composed of five dogs respectively; negative control group (NC, non-treated), positive control group (PC, 2% carboxymethyl chitosan solution treated), and experiment 1 group (E1, 0.25 ml PX/SA mixture of abraded area), experiment 2 group (E2, 0.5 ml PX/SA mixture of abraded area), experiment 3 group (E3, 1.0 ml PX/SA mixture of abraded area). Venous blood specimens were collected from all experimental animals for hematologic and biochemical analysis: WBC, fibrinogen, AST, ALT, ALP, BUN and creatinine. The anti-adhesion effect was evaluated using a serosa abrasion model. The denuded ileum was coated with PX/SA mixture, carboxymethyl chitosan solution or neither. The tensile strength of the adhesion site was evaluated with a tensiometer. For histopathological examination, tissue samples of the liver and kidney were collected from all dogs. According to the results, the frequency and tensile strength values for adhesion separation in PX/SA group were significantly lower than those in negative control group ($p < 0.05$). In E2 group, the tensile strength was significantly decreased in consideration of PX/SA dose. The values of AST, ALT, ALP, BUN and creatinine of the control and the experimental groups showed no statistical differences. No obvious microscopic differences were noted among tissue sections obtained from all groups. The results suggest that PX/SA mixture may be effective on reducing peritoneal adhesion formation in dog and that 0.5 ml PX/SA mixture of abraded area is most effective dose. Moreover, PX/SA mixture was considered not to have toxicity for the liver and the kidney.

Key words **:** adhesion, prevention, poloxamer, alginate, dog.

Introduction

Abdominal adhesions are defined as pathologic bonds between surfaces of the peritoneal cavities formed during the scarring of peritoneal surface defects. These bonds may range from a thin film of connective tissue to a thick and fibrous bridge containing blood vessels or a direct connection between two organ surfaces (6). These postoperative intra-abdominal adhesions are a significant cause of morbidity and mortality, leading to mechanical bowel obstruction, female infertility, and technical difficulty during subsequent surgical procedures (17,19). Many factors may influence the development of this adhesion, including mechanical trauma, chemical irritation, drying of the serosa, bleeding into the abdominal cavity, ischemia, infection, and foreign materials (16). Numerous techniques have been used in attempts to prevent the formation of intraperitoneal adhesion following surgical trauma to serosal or peritoneal surfaces (6,8,10,22). Anti-inflammatory drugs which were traditional adjuvants were clinically used because they may limit the release of fibrinous exudate in response to inflammation at the surgical site (4).

At present, physical barrier anti-adhesives which are forms of solution or membrane have been developed in an attempt to solve these problems. Bio-degradable physical barriers have been used to prevent adhesion formation by mechanically limiting tissue apposition during the critical period of mesothelial repair and healing (10). But, both types of tissue barriers have some limitations in their practical applications. Effect of solution adhesion barriers was reduced because they didn't stay for a long time on injury site and membrane adhesion barriers have several shortcomings that are unchangeable form, inconvenience in use and limit of applying abdominoscopy (12). To complement these demerits, a temperature-sensitive Poloxamer/Sodium Alginate/$CaCl_2$ (PX/SA) mixture which have a changeable property from a solution to gel when it was applied to the affected part was introduced as an adhesion barrier. Hydrophilic PEG component in poloxamer is known that it prevents adhesion of tissues. The gel stability of Poloxamer was improved by adding mild cross-linked sodium Alginate which was generously used a bio-degradable carrier of drugs and $CaCl_2$ mixture (12).

The aim of this study was to determine the optimal dose of PX/SA mixture for prevention of intraperitoneal adhesion formation and whether it has toxicity or not to major organs.

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Materials and Methods

Experimental groups

Twenty five healthy adult mongrel dogs (weighing 4.68 ± 1.67 kg) were used in this study. All dogs were housed in each cage, and had an initial adaption period for two weeks until the experiment was started. These dogs were divided into five experimental groups each five dogs; negative control group (NC), positive control group (PC), experiment 1 group (E1), experiment 2 group (E2) and experiment 3 group (E3).

Preparation of materials

2% carboxymethyl chitosan (Sigma, USA, CMC) solution which was verified as fine anti-adhesion material used in positive control. This solution was prepared by boiling 1000 ml of sterile water and adding 20 g of CMC powder. PX/SA mixture which was composed of 30% polyethylene glycol-polypropylene glycol-polyethylene glycol (Poloxamer®, BASF, Germany), 0.6% sodium alginate (Sodium alginate®, Sigma, USA), and 0.06% CaCl2 was applied in E1, E2 and E3 each different volume. Before use for the experiment, the 2% CMC solution and PX/SA mixture were sterilized by autoclave (121°C, 15 minutes).

Surgical procedures

The animals were premedicated with 0.05 mg/kg of atropine sulfate (Atropine®, Dai Han Pharm. Co., Korea) subcutaneously and induced with 10 mg/kg of tiletamine/zolazepam (Zoletil®, Virbac Co., France) intravenously for applying a serosa abrasion model (18,20,23). For maintenance of anesthesia, isoflurane (Airane®, Ilsung Pharm Co., Korea) was used. Then, dogs were positioned in dorsal recumbency, their ventral hair was shaved with a electric clipper and their abdominal skin was scrubbed with povidone-iodine and rinsed with 70% alcohol for aseptic surgery. A ventral mid-line incision (about 7 cm) was made through the skin, subcutaneous tissue, and peritoneum. The ileocecal junction was identified, and then all animals received standard surgical defects. Homogenous petechial hemorrhages of serosa was created about 1 × 1.5 cm area by scraping with a scalpel blade No. 10 at a distance of 5 cm from the cecum. Total five distinct surgical lesions were made at intervals of 5 cm. In negative control group, none of treatment was performed at each abrasion site. In positive control group, 1 ml/kg of 2% CMC solution was coated over the each abraded tissues and 6 ml/kg of 2% CMC solution was infused over an entire abdominal cavity before closure. PX/SA mixture was simply coated over the abraded tissues in E1, E2 and E3 groups with dose of 0.25 ml, 0.5 ml and 1 ml per abraded area, respectively. The abdominal muscles were closed by a simple continuous pattern with 3-0 polyglactin 910 (Vicryl®, Jognson & Johnson Medical Korea Ltd., Korea) and the skin was closed by a simple interrupted pattern with 3-0 nylon (BLUE NYLON®, Ailee Co. Ltd, Korea). All dogs were administered cefazolin (Cefazolin®, Chong Kun Dang Pharm Co., Korea, 20 mg/kg SC, tid) for 3 days to reduce the risk of postoperative infection.

Test items

To evaluate toxicity of PX/SA mixture, blood cell count test and blood chemistry test were performed on preoperative day and postoperative day 1, 4, 7, 14 and 21. Evaluated items were WBC (MS9-5®, MELET SCHLOESING Laboratoires, France), fibrinogen (ECLIPSE E200®, Nikon Co., Japan), AST, ALT, ALP, BUN and creatinine (SPOTCHEM SP-4410®, ARKRAY Co., Japan).

For assessing degree of postoperative abdominal adhesion, euthanasia was performed with overdosing KCl and then a postmortem examination was conducted on postoperative day 21. Sites and frequency of induced adhesions were identified in each group. After that, the adhesive site was excised to test tensile strength of the adhesion and to evaluate histologic finding. The tensile strength of the adhesion was evaluated with a tensiometer (H500DM®, Hounsfield Co., UK). Both sides of the adhesive tissue ends were fixed tightly not to slip from the clamps and pulled until the adhesive region was broken. Maximum values of tensile strength were measured.

To evaluate histologic toxicity of liver and kidney, 25 samples of the liver and kidney were collected from all experimental animals. All samples fixed in 10% neutral buffered formalin were embedded in paraffin, sectioned (3~4 μm) and stained with hematoxylin and eosin, and then the histopathological profiles of each sample were observed under light microscope (ECLIPSE E200®, Nikon Co., Japan). All observed abnormal histopathogical findings were subdivided into 3 degrees: 3+ Severe, 2+ moderate, 1+ slight.

Statistical analysis

Significant differences in the WBC, fibrinogen, AST, ALT, ALP, BUN and creatinine were evaluated using oneway analysis of variance (ANOVA) and Kruskal - Wallis H ANOVA test among the control group and the treatment groups. Frequency and tensile strength of adhesive tissue among the control group and the treatment groups were evaluated using independent t-test, independent samples test and oneway ANOVA test. All data were expressed as mean ± standard deviation (SD). P values less than 0.05 were considered to be significant.

Results

WBC

The values of WBC ($\times 10^3/mm^3$) were 14.3 ± 4.4, 10.2 ± 2.4, 14.6 ± 5.3, 13.7 ± 4.2 and 15.0 ± 3.7 on preoperative day, 23.6 ± 7.3, 23.2 ± 6.8, 19.2 ± 5.4, 13.6 ± 8.5 and 15.5 ± 6.2 on day 1, 17.0 ± 4.8, 19.6 ± 11.2, 18.6 ± 7.1, 19.9 ± 10.6 and 15.4 ± 5.1 on day 4, 18.85 ± 3.1, 25.8 ± 8.3, 16.8 ± 6.5, 20.5 ± 7.2 and 18.3 ± 8.1 on day 7, 18.3 ± 10.9, 21.3 ± 6.7, 18.6 ± 5.4, 19.8 ± 6.6 and 20.4 ± 6.2 on day 14, and 16.2 ± 7.5, 16.2 ± 6.8, 16.6 ± 8.9, 16.3 ± 6.3 and 22.4 ± 4.5 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 1). There were no significant differences between the control and experimental groups.

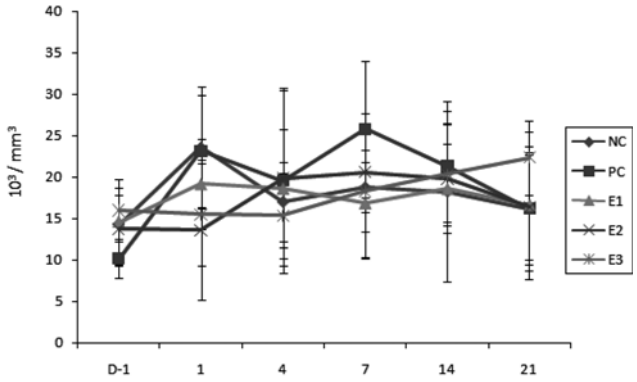

Fig 1. The changes of total WBC counts in the control and experiment groups. (Mean ± SD, $\times 10^3/mm^3$).

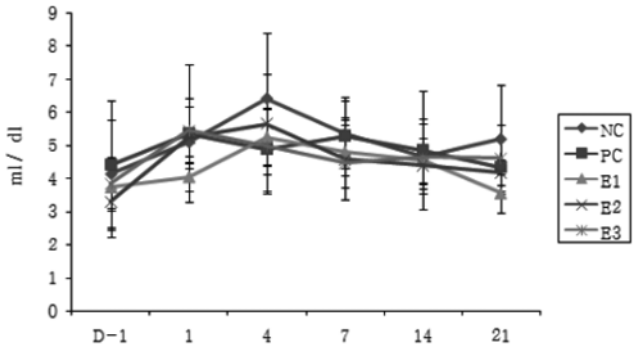

Fig 2. The changes of total fibrinogen concentrations in the control and experiment groups. (Mean ± SD, mg/dl).

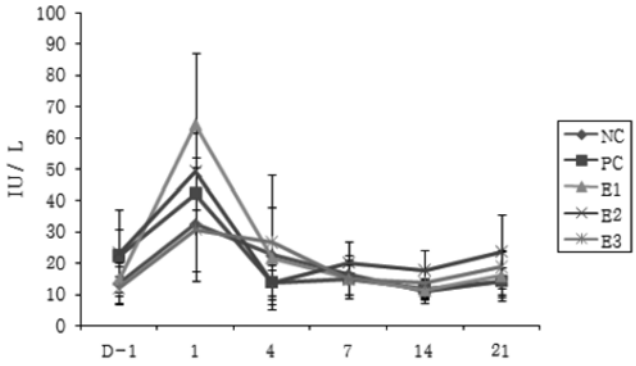

Fig 3. The changes of AST in the control and experiment groups. (Mean ± SD, IU/L).

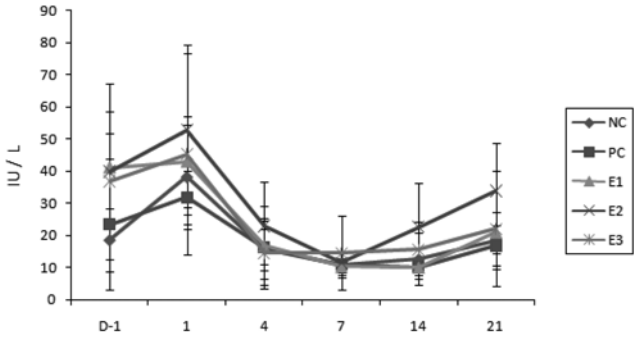

Fig 4. The changes of ALT in the control and experiment groups. (Mean ± SD, IU/L).

Fibrinogen

The values of fibrinogen concentration (mg/dl) were 4.2 ± 1.6, 4.4 ± 2.0, 3.7 ± 0.7, 3.3 ± 1.1 and 3.9 ± 0.8 on preoperative day, 5.1 ± 0.5, 5.4 ± 2.1, 4.0 ± 0.4, 5.2 ± 0.9 and 5.5 ± 1.0 on day 1, 6.4 ± 2.0, 4.9 ± 1.3, 5.2 ± 0.9, 5.6 ± 1.5 and 5.0 ± 1.4 on day 4, 5.3 ± 1.0, 5.3 ± 1.2, 4.8 ± 1.1, 4.6 ± 1.2 and 4.5 ± 1.1 on day 7, 4.7 ± 1.0, 4.9 ± 1.8, 4.6 ± 0.7, 4.4 ± 0.6 and 4.7 ± 1.1 on day 14, and 5.2 ± 1.6, 4.3 ± 0.9, 3.6 ± 0.6, 4.2 ± 0.4 and 4.6 ± 1.0 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 2). There were no significant differences between the control and experimental groups.

AST, ALT and ALP

AST

The values of AST concentration (IU/L) were 13.4 ± 6.7, 22.2 ± 15.1, 14.8 ± 4.3, 23.2 ± 7.5 and 12.0 ± 2.6 on preoperative day, 32.4 ± 18.3, 42.0 ± 11.5, 64.2 ± 22.9, 49.2 ± 12.3 and 30.6 ± 13.4 on day 1, 22.4 ± 15.5, 13.6 ± 4.2, 21.6 ± 16.3, 13.8 ± 5.5 and 26.6 ± 21.4 on day 4, 16.2 ± 6.5, 14.8 ± 6.1, 15.0 ± 5.0, 20.0 ± 6.7 and 14.6 ± 4.8 on day 7, 10.8 ± 2.5, 11.8 ± 2.8, 11.2 ± 3.9, 17.6 ± 6.5 and 13.6 ± 4.8 on day 14, and 13.8 ± 4.7, 14.2 ± 4.3, 15.8 ± 7.9, 23.6 ± 11.9 and 18.8 ± 5.3 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 3). There were no significant differences between the control and experimental groups.

ALT

The values of ALT concentration (IU/L) were 18.6 ± 9.9, 23.4 ± 20.3, 41.0 ± 17.5, 39.8 ± 27.2 and 36.8 ± 15.0 on preoperative day, 38.2 ± 16.2, 31.8 ± 8.2, 42.8 ± 14.3, 52.8 ± 26.3 and 45.2 ± 31.3 on day 1, 15.8 ± 9.4, 16.2 ± 12.8, 16.6 ± 5.5, 22.8 ± 13.9 and 14.4 ± 10.0 on day 4, 10.8 ± 4.0, 10.8 ± 4.0, 10.2 ± 1.8, 11.4 ± 3.9 and 14.4 ± 11.5 on day 7, 12.6 ± 8.1, 10.2 ± 2.7, 10.0 ± 2.2, 22.6 ± 13.7 and 15.4 ± 8.9 on day 14, and 18.2 ± 8.9, 16.8 ± 6.2, 20.8 ± 6.5, 33.8 ± 14.9 and 22.0 ± 17.9 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 4). There were no significant differences between the control and experimental groups.

ALP

The values of ALP concentration (IU/L) were 117.6 ± 109.4, 113.0 ± 85.5, 53.2 ± 8.5, 99.8 ± 45.2 and 85.0 ± 38.9 on preoperative day, 186.2 ± 63.1, 156.4 ± 36.5, 163.0 ± 61.1, 215.8 ± 31.1 and 221.6 ± 95.1 on day 1, 116.8 ± 77.0, 98.8 ± 41.5, 118.8 ± 37.5, 186.4 ± 31.5 and 136.8 ± 48.2 on day 4, 135.4 ± 49.4, 108.0 ± 32.7, 85.8 ± 17.7, 138.8 ± 19.2 and 104.0 ± 35.3 on day 7, 130.8 ± 83.1, 74.4 ± 19.9, 79.0 ± 24.1, 107.0 ± 17.4 and 91.4 ± 34.1 on day 14, and 114.4 ± 45.7, 78.4 ± 18.9, 62.8 ± 11.6, 79.2 ± 19.2 and 72.6 ± 31.1 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 5). There were no significant differences between the control and experimental groups.

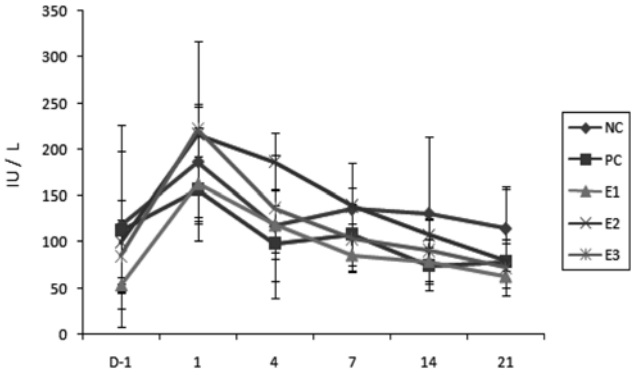

Fig 5. The changes of ALP in the control and experiment groups. (Mean ± SD, IU/L).

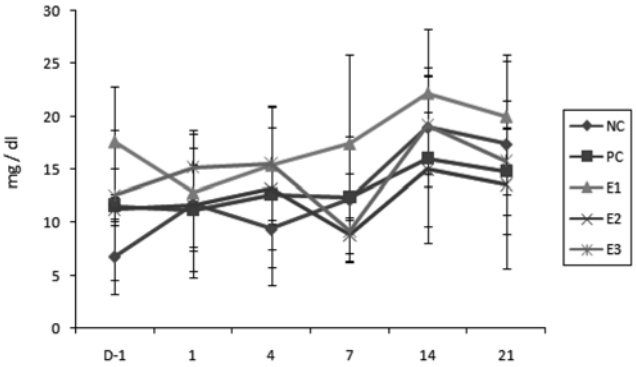

Fig 6. The changes of BUN in the control and experiment groups. (Mean ± SD, mg/dl).

BUN, Creatinine

BUN

The values of BUN concentration (mg/dl) were 6.8 ± 3.6, 11.6 ± 7.1, 17.6 ± 5.2, 11.2 ± 1.5 and 12.6 ± 2.5 on preoperative day, 11.8 ± 6.9, 11.2 ± 5.9, 12.8 ± 5.5, 11.6 ± 3.9 and 15.2 ± 3.1 on day 1, 9.4 ± 3.7, 12.6 ± 8.5, 15.4 ± 5.5, 13.2 ± 5.7 and 15.6 ± 5.4 on day 4, 12.2 ± 5.9, 12.4 ± 2.2, 17.4 ± 8.5, 8.8 ± 1.6 and 9.2 ± 2.9 on day 7, 19.0 ± 5.6, 16.0 ± 7.9, 22.2 ± 6.1, 15.0 ± 5.4 and 19.2 ± 4.6 on day 14, and 17.4 ± 8.5, 14.8 ± 4.0, 20.0 ± 5.2, 13.6 ± 7.9 and 15.8 ± 3.1 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 6). There were no significant differences between the control and experimental groups.

Creatinine

The values of Creatinine concentration (mg/dl) were 0.6 ± 0.4, 0.8 ± 0.4, 1.1 ± 0.2, 1.0 ± 0.1 and 1.1 ± 0.2 on preoperative day, 0.6 ± 0.3, 0.8 ± 0.1, 0.9 ± 0.1, 0.8 ± 0.3 and 0.8 ± 0.2 on day 1, 0.7 ± 0.4, 0.8 ± 0.3, 1.0 ± 0.1, 0.9 ± 0.1 and 0.8 ± 0.3 on day 4, 0.9 ± 0.1, 0.6 ± 0.2, 0.9 ± 0.6, 1.0 ± 0.1 and 1.1 ± 0.1 on day 7, 0.9 ± 0.1, 0.7 ± 0.3, 0.6 ± 0.1, 0.8 ± 0.3 and 1.0 ± 0.3 on day 14, and 0.9 ± 0.2, 0.8 ± 0.2, 0.7 ± 0.2, 0.6 ± 0.2 and 0.3 ± 0.1 on day 21 in NC, PC, E1, E2 and E3 group, respectively (Fig 7). There were no significant differences between the control and experimental groups.

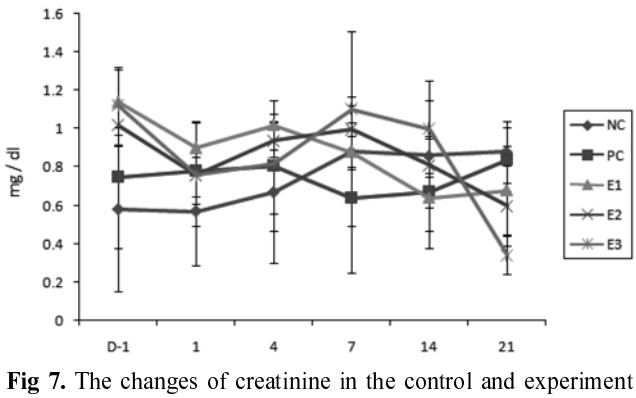

Fig 7. The changes of creatinine in the control and experiment group. (Mean ± SD, mg/dl).

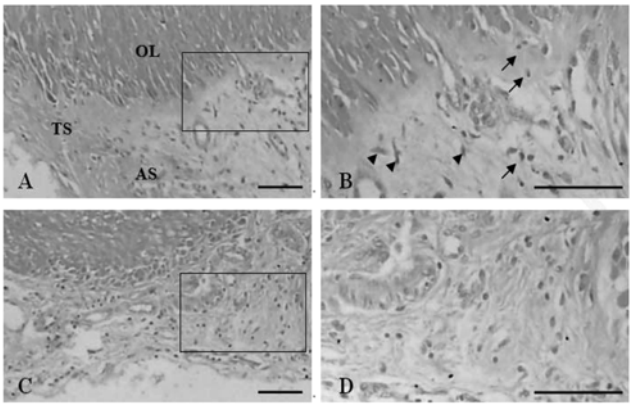

Fig 8. The representative histological profiles of adhesive spots in the ilium. Squares mean the enlarged areas in right column. All H&E stain, scale bars = 80 μm. Adhesion spots (AS) consisted of fibrotic tissues were detected in the tunica serosa (TS) of near the jejunum (A, C) with some inflammatory cells (arrows) and fibroblasts (arrow heads) invaded into the tunica serosa (B, D). OL; Outer layer of tunica muscular.

Assessment of formed adhesions

On day 21 after adhesion induction, the peritoneal adhesions were observed in serosa-serosa, serosa-mesentery, serosa-omentum and serosa-parietal peritoneum (Fig 8). To assess degree of peritoneal adhesions, the frequency of adhesion in experiment groups was compared with that in control groups (Fig 9). The frequencies of induced adhesion in serosa-serosa site were 4.4 ± 2.9, 7.3 ± 1.5, 1.0 ± 0.7, 1.0 ± 0.7 and 1.2 ± 1.1, in serosa-mesentery site were 2.4 ± 2.5, 3.3 ± 2.1, 1.8 ± 0.8, 0.6 ± 0.6 and 0.6 ± 0.5, in serosa-omentum site were 2.2 ± 1.5, 1.5 ± 1.9, 1.8 ± 1.3, 0.6 ± 0.6 and 0.4 ± 0.6, in serosa-parietal peritoneum site were 0.6 ± 1.3, 0.3 ± 0.5, 0, 0 and 0 in NC, PC, E1, E2 and E3 group, respectively. Values of adhesion frequency in experiment groups were lower than those in NC group (Fig 9). Frequency of serosa-mesentery and serosa-omentum adhesion between E1 and E2 (or E3) showed significant differences ($p < 0.05$). But frequency of serosa-serasa, serosa-mesentery and serosa-omentum adhesion between E2

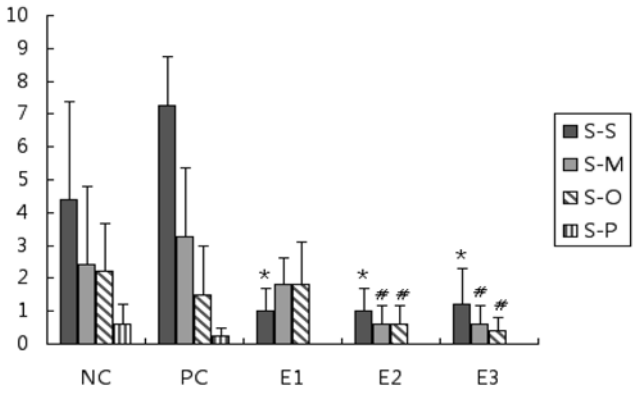

Fig 9. The frequency of formed adhesions. *p < 0.05 compare with NC group. #p < 0.05 compare with E1 group. S - S = serosa-serosa, S - M = serosa-mesentery, S - O = serosa-omentum, S - P = serosa - parietal peritoneum.

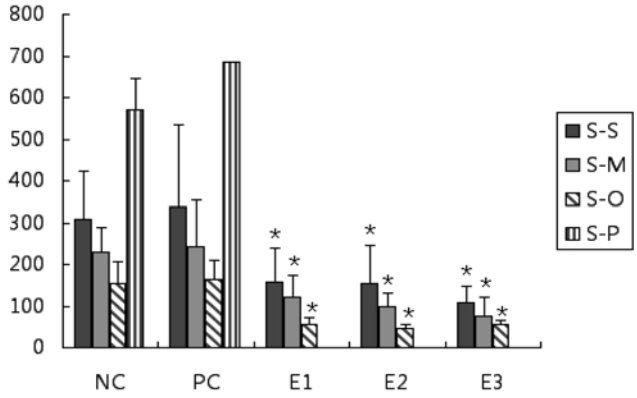

Fig 10. The tensile strengths of formed adhesions. *p < 0.05 compare with NC group. S - S = serosa-serosa, S - M = serosa -mesentery, S - O = serosa - omentum, S - P = serosa - parietal peritoneum.

and E3 showed no significant differences.

The strengths (gram force, gf) of the adhesion separation in serosa- serosa, serosa-mesentery, serosa-omentum and serosa-parietal peritoneum were evaluated to compare a severity of adhesion. The tensile strengths of formed adhesion in serosa-serosa site were 309.2 ± 113.9, 339.2 ± 196.3, 157.2 ± 81.0, 152.6 ± 94.3 and 107.2 ± 38.7, in serosa-mesentery site were 228.7 ± 60.9, 241.2 ± 111.6, 122.2 ± 51.7, 99.3 ± 30.8 and 76.0 ± 45.9, in serosa-omentum site were 153.4 ± 53.3, 164.2 ± 44.9, 55.3 ± 17.0, 46.3 ± 9.1 and 54.5 ± 10.6, in serosa-parietal peritoneum site were 549.0 ± 75.6, 684.0 ± 0.0, 0, 0 and 0 in NC, PC, E1, E2 and E3 group, respectively (Fig 10).

The tensile strengths of the experiment groups were significantly low ($p < 0.05$) in comparison with that of the control group in all sites excepting serosa-parietal peritoneum region. Tensile strength of all adhesion site among experimental groups showed no significant differences.

Histologic evaluation of the liver and kidney

Abnormal histopathological changes were not detected in all three experimental groups as compared with NC and PC groups (Table 1). No abnormal changes in kidney were detected in NC (A,B) and PC (C,D) , E1 (E,F), E2 (G,H) and E3 (I,J) group (Fig 11). No abnormal changes in the liver were detected in NC (A~D) and PC (E~G), E1 (H,I), E2 (J,K) and E3 (L~N) respectively except for some accidental findings; Slight congestions of the liver were detected in 1 dog (1/5; 20%) of NC and E3, 2 dogs (2/4; 40%) of PC, E1 and E2 group, respectively. Slight focal inflammatory cell infiltrations (asterisks) were restrictly detected to 1 dog (1/5; 20%) of NC, 1 dog of PC and 1 dog of E3, respectively. Slight hemosiderin depositions (arrows) in congestional regions were restrictly detected to only 1 dog (1/5; 20%) of negative control as accidental findings (Table 1, Fig 12).

Discussion

Injury or inflammation of a serosal surface generally initiates adhesion formation processes. Destroyed peritoneum fails to absorb fibrinogen-rich fluid exudates. In addition, activation of inflammatory cells, secretion of inflammatory cytokines, and activation of the complement-coagulation cascades creates thrombin, which converts fibrinogen to fibrin in the exudates (4,5,7,21). The formation of adhesion 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 disappears, macrophages become the predominant leukocyte and a larger number of fibroblasts and associated collagen are present. On day 5, small vascular channels containing endothelial cells are seen, and days 5 through 10, fibroblasts become aligned within the adhesion while collagen deposition and organization advance (1-4).

The clinical consequences of these adhesion are serious. Innumerable substances and methods have been used, either locally in the peritoneal cavity or systematically, to prevent or reduce the occurrence of postoperative peritoneal adhesion (6,10). This may reduce the amount of exudates, prevent its coagulation, reduce contact between surfaces, and remove fibrin after its appearance and stop proliferation of fibroblasts (9). Recent efforts to lower the incidence of adhesion formation have been focused on a barrier. Because placing a barrier between these wounded surfaces can prevent postoperative peritoneal adhesion, numerous natural and synthetic resorbable barriers have been shown to reduce adhesion formation.

Anti-adhesion materials of solution or membrane type which played a role in preventing intraperitoneal adhesion as a physical barrier have been used practically (22). However, solution materials can not be concentrated in surgery site and spread over peritoneal cavity, because they don't have a fixed form. Also, they have characters which are subject to be absorbed and degraded easily. Membrane materials have some shortcomings which are poor transformation, and difficulty of applying abdominoscopy. In the present study, PX/SA mixture which is temperature-sensitive transformational anti-adhesion material was used. When surrounding temperature rise to 25°C

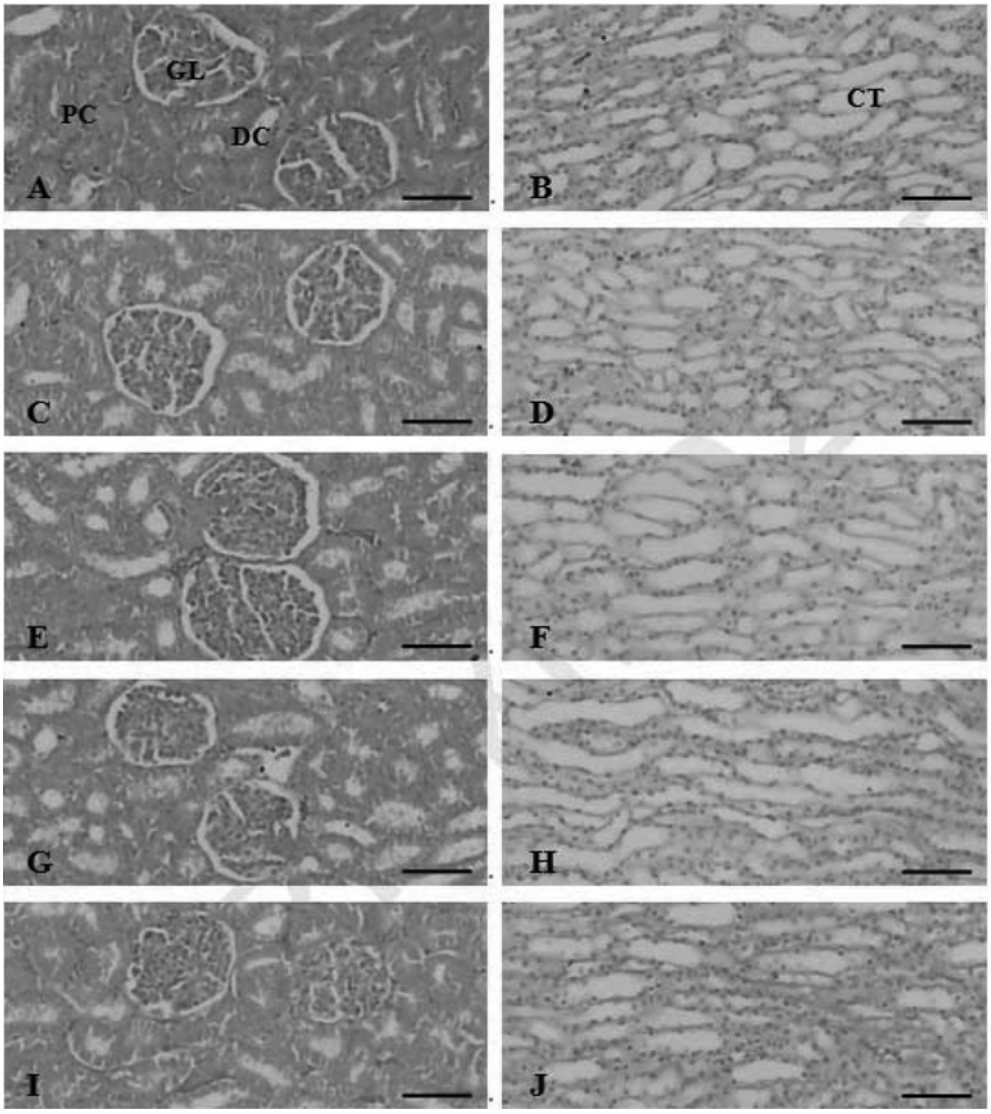

Fig 11. The representative histological profiles of kidney. GL, glomerulus; DC, distal convoluted tubule; PC, proximal convoluted tubule; CT, collecting tubule. All H&E stain, scale bars = 80 μm. A,C,E,G & I; cortex and B,D,F,H & J; medulla.

Table 1. The representative histological profiles of kidney of study groups. Values are expressed as observed animals/total observed animals

Group	Control groups		Experimental groups		
	Negative	Positive	E1	E2	E3
Kidney					
Normal	5/5	5/5	5/5	5/5	5/5
Liver					
Normal	3/5	2/5	3/5	3/5	3/5
Congestion	1/5	2/5	2/5	2/5	1/5
HD*	1/5	0/5	0/5	0/5	0/5
IF*	1/5	1/5	0/5	0/5	1/5

* Abbreviation of lesions - HD, hemosiderin deposition; IF, focal inflammatory cell infiltration

reach, PX/SA mixture is changed from solution form to gel form. In other words, if PX/SA mixture in liquid stats was injected at surgery region, it was immediately transformed to semi-liquid stats in injected site. PX/SA mixture was com-

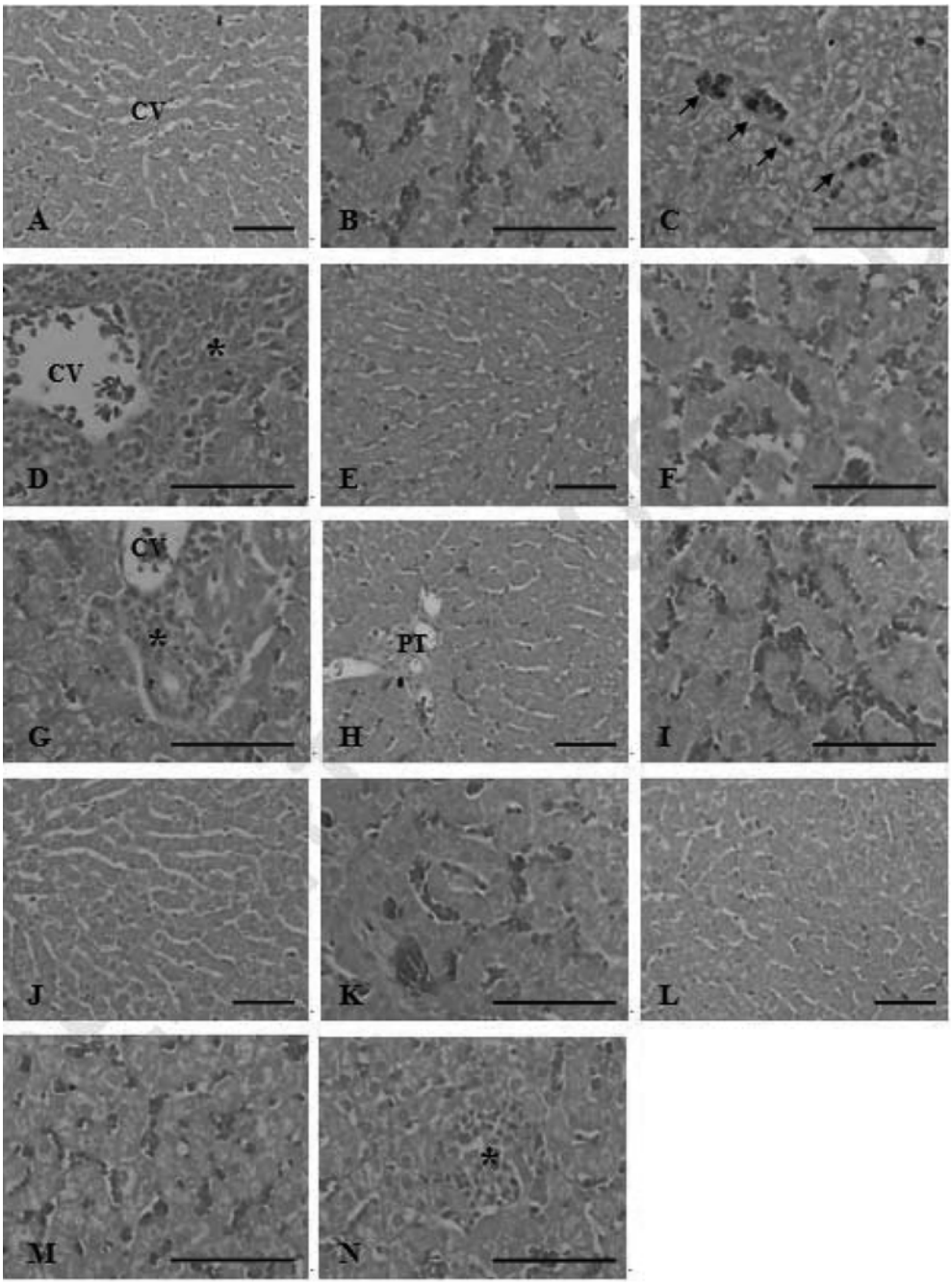

Fig 12. The Representative histological profiles of liver of study groups. CV; central veins, PT; portal triad. All H&E stain, scale bars = 80 μm.

posed of a bridge with poloxamer, sodium alginate and $CaCl_2$ has elevated stability that amount more than 60% of initial injected volume existed in 7 days and it was completely exhausted in 21 days (12). The PX/SA mixture was applied to E1, E2 and E3 group as anti-adhesion material.

The PC group which was applied with CMC solution well known as a liquefied anti-adhesion was showed higher frequency and intensity of adhesion than the NC group did. These were considered an error of experimental process. In comparison among five groups, the experimental groups were showed much lower frequency of adhesion than those of NC group. Also, the tensile strengths of adhesion region in the experiment groups were also remarkably low than that of NC group. The most favorable prevention of postoperative intraperitoneal adhesion among the experiment groups was achieved in the E2 group ($p < 0.05$).

Because there were no significant changes of WBC and fibrinogen between the control groups and the experiment groups, it was regarded that PX/SA mixture has no hematological toxicity. Also there were no significant differences

among all groups in AST, ALT, ALP, BUN and creatinine. It represented that there were no toxicity of PX/SA mixture to liver and kidney. Only accidental findings such as slight [1+] congestion, deposition of hemosiderin and focal inflammatory cell infiltration in liver were sporadically detected throughout the whole experimental groups including both negative and positive controls. The congestions of liver detected, they were considered as results from difficult or bad exsanguinations not test material treatment related toxicological signs. Similar frequent congestions were also detected in negative and positive controls. These results are considered that PX/SA mixture did not induced any histopathological changes on liver and generally, focal inflammatory cell infiltration in liver was rarely observed accidental findings in animals (13,14). The deposition of hemosiderin pigments is an iron-containing, golden-brown, grandular pigment derived from ferritin, the primary iron storage protein. Although some chemical drugs like cefodizime sodium induced hemosiderin depositions in liver (11), most hemosiderin in Kupffer cells and other macrophages located in liver is derived from breakdown of erythrocytes (15). However, in this study, they were located in congestional regions restricted to one dog of negative control, not Kupffer cells or macrophages; therefore, it is simply considered as secondary changes related to congestion.

Conclusion

The results suggest that PX/SA mixture may be effective on reducing peritoneal adhesion formation in dog and that 0.5 ml PX/SA mixture of abraded area is most effective dose. Moreover, PX/SA mixture was considered not to have toxicity for the liver and the kidney.

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개에서 Poloxamer/Sodium Alginate 혼합물의 용량에 따른 복강 유착방지 효과

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요 약 : 이 실험은 복강 유착 방지효과를 나타내는 Poloxamer/Sodium Alginate (PX/SA) 혼합물의 최소용량과 주요기관의 독성 여부에 대해 알아보기 위해 실시하였다. 건강한 잡종성견 25마리를 음성대조군 (무처치), 양성대조군 (2% carboxymethyl chitosan 용액 처치), 실험군 1 (PX/SA 혼합물 0.25 ml 처치), 실험군 2 (PX/SA 혼합물 0.5 ml 처치), 실험군 3 (PX/SA 혼합물 1.0 ml 처치) 으로 나누고 각 군당 5마리씩 배치하였다. 혈액학 검사 (백혈구,섬유소원)와 혈액화학 검사(AST, ALT, ALP, BUN, Creatinine)를 위해 정맥에서 혈액을 채취하였다. 유착방지효과를 알아보기 위해 돌창자에 찰과상을 일으켜 carboxymethyl chitosan 용액, PX/SA 혼합물을 처치하는 장막 찰과 모델을 이용하였다. 유착부위의 유착강도는 장력측정기를 이용하여 측정하였다. 조직검사를 위해 각 군의 모든 개로부터 간과 신장 조직을 채취하였다. PX/SA혼합물을 처치한 실험군이 음성 대조군보다 유착발생 빈도와 유착강도 모두 낮게 측정되었다. 실험군 간의 비교에서 실험군 2에서 유착강도가 유의적으로 감소하였다. AST, ALT, ALP, BUN, Creatinine 은 대조군과 실험군 사이 유의적 차이가 발견되지 않았으며, 모든 군에서 얻어진 조직 표본에서도 군간 유의적 차이를 보이지 않았다. 본 실험의 결과, PX/SA 혼합물 0.5 ml는 복강 유착 형성을 효과적으로 감소시켰으며, 물질이며, 혈액 및 주요 장기에 대한 독성도 없는 것으로 사료된다.

주요어 : 유착, poloxamer, alginate, 개.