J Vet Clin 18(3) : 257-264(2001)

Effect of Chitosan-Trimer on the Prevention of Postoperative Intraperitoneal Adhesion Formation in Rats

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Abstract : This study was performed to investigate the effects of chitosan-trimer (CT) on the prevention of postoperative adhesion formation in the rat model. All animals divided into PBS (control), 1% CT, 3% CT, and chitin treated group. The mean adhesion score in 1% CT group (1.03±0.63), 3% CT group (0.64±0.53) and chitin group (1.67±0.71) was found to be lower than that in control group (2.07±0.81). More favorable adhesion prevention was achieved in 3% CT group (0.64 ±0.53) in comparison with the control group, 1% CT group, and chitin group without any hemorrhagic complications. A statistically significant difference was observed in adhesion formation between control group and 3% CT group ($p<0.001$). In control group, 44 of 45 sites (97.7%) formed adhesions between the intestine defects. In contrast, 3% CT was effective in reducing the incidence of adhesion formation to 17 of 45 sites (62.2%) ($p<0.05$). The locations of adhesions were observed in serosa-serosa (60%), serosa-mesentery (13.3%), serosa-connective tissue of testis (10%), omentum-liver (10%), serosa-omentum (3.3%), serosa-cecum (3.3%), and serosa-incision (0%). On the results of histological analysis, grade of inflammation and fibrosis at the sites of postoperative peritoneal adhesion formation were not significantly different in all groups. But, 3% CT showed the lowest score of inflammation and fibrosis. In 3% CT group, the rate of increase of plasma fibrinogen was significantly lower compared with that in control group from pre-operation to 10 days later ($p<0.05$). There were no appreciable difference in the CBC, leukocyte differential counts and total protein concentrations among four groups. In conclusion, our data suggested that CT should be effective on reducing adhesion formation in experimental rat models. The results also showed that 3% CT does not adversely affect normal wound healing and healthy recovery after operation.

Key words **:** Intraperitoneal adhesion, Chitosan-Trimer, Fibrinolytic agent, Plasma fibrinogen

Introduction

Postoperative intraperitoneal adhesion formation is considered to be an inevitable complication after surgical operations. Abdominal adhesions are defined as pathologic bonds between surfaces of the peritoneal or pelvic cavities formed during the scarring of peritoneal surface defects[10]. Adhesions typically form between defects such as a damaged peritoneal surface that is no longer covered by normal mesothelium and any other tissue that it comes in contact with, such as normal or damaged peritoneum or ovaries[10].

Major complications include infertility[22], intestinal obstruction, and recurrent abdominopelvic pain[2,25]. In addition, intraperitoneal adhesions make subsequent abdominal operation more difficult and potentially hazardous.

An inflammatory exudate forms following peritoneal injury and leads to the depositon of fibrin in the peritoneal cavity[23]. Peritoneal injury initiates an inflammatory response, followed by an increase in permeability and release of fibrin-rich exudate[1]. Dogs and cats have an active fibrinolytic mechanism within their peritoneal cavities[7,23]. This prevents the development of serious restrictive fibrous adhesions by decreasing the amount of fibrin adhering to various surfaces[1]. Fibrinolytic mechanism is stimulated naturally by plasminogen activating substances that are present in mesothelial cells and submesothelial blood vessels[4,8,9,28,30]. If not quickly removed by fibrinolysis, the fibrin deposition produces dense, permanent adhesion formation[4].

Until now, numerous agents have been tested in animal and clinical studies as to their ability to prevent or reduce the incidence of intraperitoneal adhesion[2,5,10,25]. Recent efforts to lower the incidence of adhesion formation have been focused on a barrier and a fibrinolytic agent. Hyaluronic acid(HA) is a linear polysaccharide comprised of alternating glucosamine residues that interact with other proteoglycans to provide stability and elasticity to the extracellular matrix of all tissues[16]. HA is a high-weight polysaccharide found through mammalian tissues and is an important component of the extracellular matrix involved in tissue repair[13]. HA has been known as playing a role in normal wound healing[3,16] and in inhibiting peritoneal[5,9,13,21,29], pericardial[24] adhesion formation. Especially, HA acts as a barrier to separate damaged tissue surfaces during the critical phases of peritoneal wound repair and was associated with a significant reduction in postoperative intraperitoneal adhesions. But, HA has proved unattractive in a clinical use because of its high cost and limited efficacy[29].

Chitosan Trimer(CT) is prepared by hydrolysis of chitosan from crab shell. The oligomer is composed of β-1,4-linked D-glucosamine. Oligomers are confirmed by HPLC. CT is a derivative of chitin, which is a water-soluble, hydrophilic, lubricious, and viscoelastic material[15]. CT is a novel agent with a structure similar to HA[18]. Chitosan's availability in a

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variety of useful forms including solutions, powders, flakes, gels, and films together with its unique chemical and biological properties make it a very versatile biomaterial. Many studies that chitosan accelerated wound healing in many clinical cases have already been reported in Japan[26]. But, to date, there are few reports on the effects of chitosan in the prevention of postoperative adhesion formation.

In 1996, Kennedy et al[15] documented the scientific basis for the utility of the N, O-carboxymethyl chitosan(NOCC) in the prevention of postoperative adhesion formation. They hypothesized that NOCC form a barrier to the activation of the normal pathways of fibrin deposition, and this prevent adhesions but do not affect normal wound healing. But the mechanism of action of NOCC is still unclear.

Because many of the cells and events involved in adhesion formation are also involved in the normal wound healing process, it is important to ascertain whether CT interferes with wound healing.

Therefore, in this study, the effects of chitosan-trimer on the prevention of postsurgical adhesion formation and whether CT interferes with wound healing were investigated in the rat model.

Materials and Methods

Chemicals

Chitotriose Hydrochloride used in this study was white powder (Chitosan Trimer , Seikagakukokyo Inc., Japan). Chitin powder was supplied by Sigma. Chitosan Trimer (CT) and chitin was sterilized with ethylene oxide gas and was dissolved in 2 ml of aseptic Phosphate Buffer Solution (PBS, pH 7.4) at room temperature.

Animals

51 male Sprague-Dawley rats weighing 200 to 240 g were used in this investigation. These animals were purchased from Clear Japan Inc., and had free access to a standard pellet diet and tap water. Experiments were started after an initial adaption for about one week. This research was carried out under the guidelines of animal use and care approved in Department of Veterinary Medicine, Hokkaido University, Japan. The animals were randomized into four groups. The rats of control group were treated with 2 ml of PBS solution. The rats of the other groups received different substances. The groups were described as follow:

① Control group : Group treated with 2 ml of PBS

② 1% CT group : Group treated with 2 ml of 1% Chitosan Trimer

③ 3% CT group : Group treated with 2 ml of 3% Chitosan Trimer

④ Chitin group : Group treated with 2 ml of 3% Chitin

Surgical techniques

(1) First celiotomy for induction of abrasion

The rats were anesthetized with 50 mg/kg of intraperitoneal sodium pentobarbital (Nembutal®, Dainippon Seika Kaisha Ltd., Japan). The ventral abdomen was clipped with electric shears, and the abdominal skin was disinfected with 70% alcohol and iodine solution. Operation was performed under sterile condition. After weighing, shaving, and disinfection, rats were placed in dorsal recumbency and covered with a sterile fenestrated drape. By use of aseptic technique a routine midline laparotomy was performed beginning approximately 3 cm posterior to the xiphoid process and extending caudally 3 cm in length, then skin was laterally retracted and 2 cm midline abdominal wall incision was made. After ileocecal junction was identified, the first abrasions were made on the serosal surface of ileum at 3 cm proximal to the ileocecal junction. Ileal serosa was scraped 20 times with a scalpel, rendering the serosal surface disrupted and hemorrhagic (Fig 3). Total abrasions were 3 places at intervals of 3cm and were created over a $5mm^2$. The ileal defects were exposed to air for 10 minutes. The defects were then dropped back into the abdomen, the peritoneum was closed by continuous suture of 4-0 polyglactin 910 (Coated Vicryl®, sterile, Ethicon Inc.). The skin was closed by a simple interrupted suture of 4-0 polyamide (Nurolon®, sterile, Ethicon Inc.). Each PBS, CT, and chitin was administrated as 2 ml of sterile solution before closure of peritoneum. The duration of the operation from skin incisions to the final suture was approximately 30 minutes.

(2) Second celiotomy for adhesion evaluation

Ten days after induction of abrasions all animals were anesthetized by intraperitoneal injection of sodium pentobarbital. And the second laparotomy was performed. After adhesion evaluation and blood collection for analysis, all animals were euthanized in order to gain histological samples.

Evaluation

(1) Adhesion score

An evaluator, who was blind to the group assignment, scored adhesions according to the following scale ranging from 0 to 3. The adhesion grade was determined according to Jenkins's scoring system[12] : 0 = No macroscopic adhesions; 1 = Gentle blunt dissection required to free adhesions, filmy, avascular; 2 = Aggressive blunt dissection required to free adhesions, vascularity; 3 = Sharp dissection required to free adhesions, dense, marked adhesion

(2) The incidence and location of adhesion formation

The location and incidence of adhesion formation in ileum were checked on ten days after induction of abrasions in all animals.

(3) Plasma fibrinogen

The blood was obtained from tail vein before first celiotomy and the heart before second celiotomy, and put in a

tube containing sodium citrate. Plasma fibrinogen was determined by Miller's method.

(4) Complete blood count and total protein

The blood obtained from tail vein and heart, was put in a tube containing ethylenediaminetetraacetic acid disodium salt (EDTA; 2 mg/ml of blood). Using blood treated with EDTA, the erythrocyte counts, total platelet, and white blood cell counts were performed with Celltac α MEK-6258 (Nihon Kohden Co. Ltd., Japan), and the hematocrit (Hct) value was determined by a microhematocrit method. Total protein was determined by a refractometer method.

(5) Differential counts of white blood cells

After EDTA-treated blood was fixed with ethanol, the blood smear was stained with Giemsa solution (pH 6.4) for 20-30 minutes. The differential counts of white blood cells on the blood smear were taken under microscopy.

(6) Histological observation and inflammation grading scoring

Specimens were taken from representative animals of each group and submitted for histologic analysis. The consulting pathologist was blinded as to the identity of the specimens. Specimens were fixed in 10% buffered formalin, dehydrated and embedded in paraffin. Sections (2~3 μm) were routinely stained with hematoxylin-eosin(H&E). The inflammation and fibrosis grade were evaluated according to Hooker's scoring system[11]. Inflammation grading scale: 0 = no; 1 = Giant cell, occasional scattered lymphocytes and plasma cells; 2 = Giants cell with increased numbers of admixed lymphocytes, plasma cells, eosinophils, neutrophils; 3 = Many admixed inflammatory cells, microabscesses present. Fibrosis grading scale: 0 = no; 1 = minimal, loose; 2 = moderate; 3 = dense

(7) Statistical analysis

This research was designed to test the hypothesis that CT reduce the incidence, extent, and severity of postoperative formation of adhesions compared with the control group. All data are expressed as mean ± standard deviation. Statistical analysis was carried out using Student's t-test and ANOVA.

Results

Forty-nine rats survived until date of second laparotomy

Adhesion score

The scores recorded for control, 1% CT, 3% CT and chitin group, indicate the severity of the experimental adhesions achieved with this model.

The mean adhesion scores in 1% CT group (1.03 ± 0.63), 3% CT group (0.64 ± 0.53) and chitin group (1.67 ± 0.71) were found to be lower than that in control group (2.07 ± 0.81). More favorable adhesion prevention was achieved in 3% CT group (0.64± 0.53) in comparison with control group, 1% CT group, and Chitin group without any hemorrhagic complication. A statistically significant difference was observed in adhesion formation between control group and 3% CT group ($p < 0.001$, Fig 1).

Control rats were found to have numerous thick, vascular adhesion. In contrast, 3% CT group was demonstrated to have excellent healing of the ileal serosa. One rats had no macroscopic adhesion formation. Although the adhesion formation was also reduced in 1% CT group compared with control group, 1% CT group was not effective than 3% CT group. When chitin was injected, chitin was not entirely absorbable because it's particle was too much big.

The incidence and location of adhesion formation

In control group, 44 of 45 sites (97.7%) formed adhesions between the intestine defects. In contrast, 3% CT was effective in reducing the incidence of adhesion formation to 17 of 45 sites (62.2%) (Table 1).

Adhesions resulting from ileum abrasion were typically between the serosa and serosa of the small bowel. The locations of adhesions were observed in serosa-serosa (60%), serosa-mesentery (13.3%), serosa-connective tissue of testis (10%) omentum-liver (10%), serosa-omentum (3.3%), serosa-cecum (3.3%), and serosa-incision (0%) (Fig 2).

Plasma fibrinogen

In 3% CT group, the rate of increase of plasma fibrinogen was significantly lower compared with control group from pre-operation to 10 days later ($p < 0.05$, Table 2).

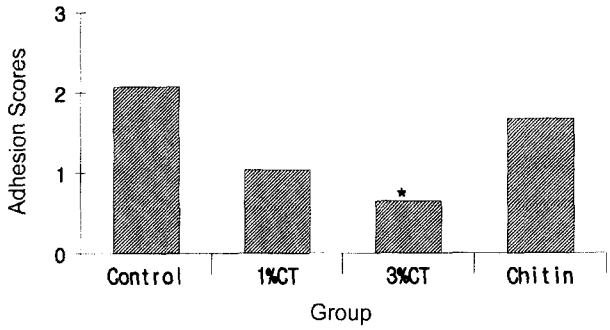

Fig 1. Postoperative adhesion scores in rats on the 10th day after operation Data were represented as mean± standard deviation. *P<0.001, 3% CT group compared with the control group.

Table 1. The incidence of adhesion formation in rats on the 10th day after operation

Groups	No. of adhesion/total no. of sites (%)
Control	44/45 (97.7%)
1% CT	27/33 (81.8%)
3% CT	28/45 (62.2%)*
Chitin	28/30 (93.3%)

*P<0.05, 3% CT group compared with all groups

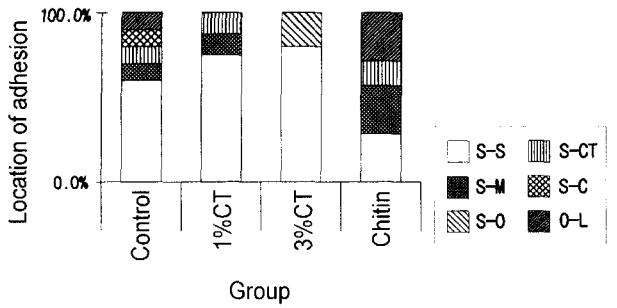

Fig 2. Postoperative location of adhesion in the ileum of rats on the 10th day after operation. *Location of adhesion: S-S = Serosa-Serosa; S-M = Serosa-Mesentery; S-O = Serosa-Omentum; S-CT = Serosa-Connective tissue of Testis; S-C = Serosa-Cecum; O-L = Omentum-Liver

Complete blood counts

Hematologic values were monitored before celiotomy and 10 days later. Hematologic values remained generally within normal ranges in all groups (Table 3, 4, 5, 6 and 7).

Differential counts of white blood cells

Differential counts of white blood cells was not significantly differences among blood smear sections from all groups. Ten days later, lymphocytes have a tendency to increase in control and chitin group.

Histologic analysis

Grade of inflammation at the sites of experimental postoperative peritoneal adhesion formation was not significantly different between 3% CT and control group (1.27 ± 0.16 vs 1.71 ± 0.17, $P > 0.05$). Similarly, Four groups had no significant differences in grade of inflammation: control group = 1.89 ± 0.11; 1% CT group = 1.56 ± 0.09; 3% CT group = 1.44 ± 0.2; chitin group = 1.77 ± 0.13

The grade of fibrosis did not differ among control (2.21 ± 0.16), 1% CT (1.89 ± 0.09), 3% CT (1.79 ± 0.90) and chitin (2.17 ± 0.10). But, 3% CT group showed the lowest score of fibrosis and inflammation (Fig 3, 4, 5 and 6).

Discussion

This study has demonstrated the efficacy of Chitosan-Trimer(CT) solution and appropriate concentration of CT on the prevention of postoperative adhesion formation in the experimental rat model. We have compared anti adhesive effects when PBS, CT or chitosan was used in the damaged small bowel. In the abrasion models, two animals, one in

Table 3. The changes of total leukocyte counts in rats induced intraperitoneal adhesion (Mean ± SD, $\times 10^3/\mu l$)

Groups	Pre-operation	10 days later
Control	9.38±0.61	13.58±4.47
1% CT	6.27±0.90	12.00±2.05
3% CT	7.10±2.05	8.43±3.04
Chitin	5.30±0.27	7.77±1.72

Table 4. The changes of total erythrocyte counts in rats induced intraperitoneal adhesion (Mean ± SD, $\times 10^6/\mu l$)

Groups	Pre-operation	10 days later
Control	7.35±0.50	6.75±0.37
1% CT	6.37±0.97	6.79±0.46
3% CT	7.02±0.45	6.77±0.48
Chitin	6.35±0.24	6.60±0.31

Table 5. The changes of hematocrit in rats induced intraperitoneal adhesion (Mean ± SD, %)

Groups	Pre-operation	10 days later
Control	44.75±0.50	42.00±2.45
1% CT	41.67±2.89	41.33±2.08
3% CT	43.00±2.94	41.50±2.65
Chitin	39.53±2.00	40.00±1.00

Table 6. The changes of total platelet in rats induced intraperitoneal adhesion (Mean ± SD, $\times 10^3/\mu l$)

Groups	Pre-operation	10 days later
Control	901.75±158.93	827.50±83.89
1% CT	866.67±62.51	763.33±23.29
3% CT	831.00±39.60	837.50±77.38
Chitin	796.00±91.66	741.00±164.15

Table 7. The changes of total protein concentration in rats induced intraperitoneal adhesion (Mean ± SD, g/dl)

Groups	Pre-operation	10 days later
Control	6.85±0.83	6.10±0.12
1% CT	7.07±0.90	6.07±0.11
3% CT	6.75±0.96	6.00±0.00
Chitin	7.53±1.71	6.07±0.12

Table 2. The changes of plasma fibrinogen concentration in rats induced intraperitoneal adhesion (Mean ± SD, g/dl)

Groups	Pre-operation	10 days later	Rate of increase of P.F
Control	118.25±16.38	192.2±20.57	80.50±9.81
1% CT	121.25±13.45	156.5±46.26	35.25±8.81
3% CT	117.5±12.18	131.00±26.89	13.50±14.71*
Chitin	120.75±11.18	165.8±40.65	45.05±19.48

*$p < 0.05$, 3% CT group compared with control group

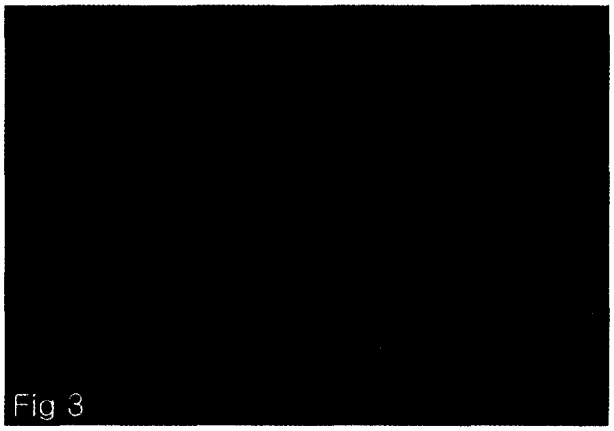

Fig 3. Histological view of small bowel 10 days after operation in a rat treated with PBS. The section from specimen shows that each outer longitudinal smooth muscle is conjugated and tunica serosa is not observed. Massive scar formation bridges one tunica muscularis and the other in control group. Fibrin deposition, neovascular structures and inflammatory cells including lymphocytes are found in gap of smooth muscle fibers (H&E stain × 100).

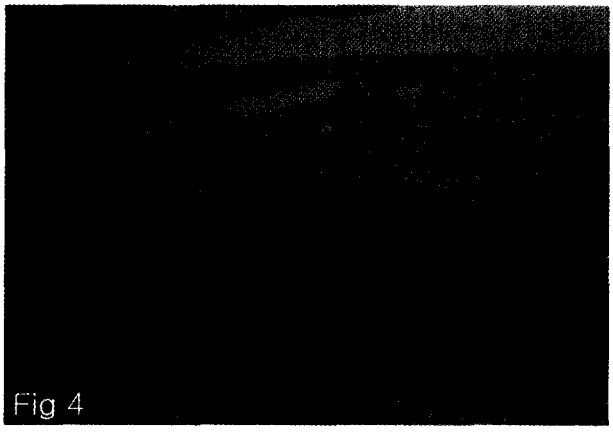

Fig 4. Histological view of small bowel 10 days after operation in a rat treated with 1% CT. The section from specimen shows that serosal area and surrounding connective tissue have inflammatory cell infiltration and neovascular structures (H&E stain × 40).

Fig 5. Histological view of small bowel 10 days after operation in a rat treated with 3% CT. The adhesion between one ileal segment and the other consists of a very small band of connective tissue (H&E stain × 40).

Fig 6. Histological view of small bowel 10 days after operation in a rat treated with chitin. In this specimen, fibroblastic proliferation infiltrates into tunica muscularis. Several inflammatory cells and neovascular structures are found (H&E stain × 40).

control group and the other in chitin group, showed severe weight loss and debility, and was required euthanasia. Based upon the results of necropsy, causes of weight loss and debility included small bowel obstruction and perforation. Thus, These animals excluded from analysis because their peritonitis was too severe to measure adhesion scores.

The evaluation of adhesion formation included the adhesion grade ascribed by Jenkins's scoring system, incidence of adhesion formation, histological observation, and hematological examination. When 3% water soluble CT was treated in the small bowel abrasion models, the result showed the 3% CT could decrease the adhesion score, incidence of adhesion formation, and the rate of increase of plasma fibrinogen than any other groups. On the results of histological analysis, grades of inflammation and fibrosis at the sites of postoperative peritoneal adhesion formation were not significantly different in all groups. But, the rat in 3% CT group showed the lowest score of inflammation and fibrosis. Although 1% CT also reduce the adhesion formation compared with PBS, 1% CT was not effective than 3% CT.

Although 3% CT was not completely prevent postoperative adhesion formation, the result of this study showed that 3% CT could significantly reduce the incidence of adhesion sites on adhesion formation than any other agent($p < 0.05$). At the time of second celiotomy this agent was completely absorbed.

Adhesions are formed when the parietal or visceral peritoneum is damaged and the basal membrane of the mesothelial

layer is exposed to the surrounding tissues1. This injury to the peritoneum, because of surgery, infection, or irrigation, causes a local inflammatory reaction, which leads to the formation of a serosanguineous exudate that is rich in fibrin[4,10]. The fibrious exudate is a part of the hemostatic process and facilitates and new blood vessels[10]. On the one hand, this deposition of fibrin is an essential component of normal tissue repair, but on the other hand, resolution of this fibrin deposit is required to restore the preoperative conditions or conditions before inflammation[10]. The dissolution of fibrin is mediated by the fibrinolytic system. In this system, the inactive proenzyme plasminogen is converted into active plasmin by tissue plasminogen activator (t-PA) or urokinase plasminogen activator (u-PA). This plasmin degrades fibrin, the matrix structure of fibrinous adhesions. When the fibrinolytic capacity is insufficient, deposited fibrin may persist and fibrinous adhesions may develop. The fibrinous adhesion become organized, characterized by deposition of collagen and concomitant vascular in growth. As a result, the adhesions are changed into fibrous, permanent adhesions[10]. Thus, an imbalance between fibrin deposition and fibrin dissolution is the key event in adhesion formation.

The results of this study showed that in 3% CT group, a rate of increase of plasma fibrinogen was significantly lower as compared with control group from pre-operation to 10 days later ($p < 0.05$). This result is similar to Kang's report[14] that plasma fibrinogen of control group continuously increase in the adhesion induced rat model. Therefore, plasma fibrinogen may be a potential indicator for degree of adhesion formation.

Macroscopically, adhesions in 3% CT treated animals were generally filmy and avascular, being severe only in 37.8% of case. Control rat showed thick and widespread adhesions in 67% of cases. Chitin and 1% CT treated animals also showed thick and vascular adhesions in $>50\%$ of cases. The locations of adhesion were observed in serosa-serosa (60%), serosa-mesentery (13.3%), serosa-connective tissue of testis (10%), omentum-liver (10%), serosa-omentum (3.3%), serosa-cecum (3.3%), serosa-incision (0%).

Histologic analysis revealed 3% CT did not induce a specific inflammatory reaction, and caused lower fibrotic response compared with the control group.

Lymphocytes have a tendency to increase in control and chitin group because of early inflammatory response. The absence of an appreciable difference in the CBC, leukocyte different counts and total protein values among four groups might demonstrate that the values of peritoneal blood were not altered in the presence of chitosan. These results indicated that anti adhesion action of CT was not degraded by leukocytes. This reduces the risk that the leukocytes will cause incidental injury to contacting normal peritoneum and actually induce adhesion formation.

Hyaluronic acid that is a novel agent with a structure similar to chitosan, a natural glycosaminoglycan, has been shown to play a role in normal wound healing[3,16] and in inhibiting pericardial[24] and peritoneal[5,9,13,21,29] adhesion formation. But, the most recently introduced HA that was not cross-linked was not effective compared with cross-linked HA[18,19,27]. Becker *et al*[2] reported that the use of hyaluronic acid-carboxymethylcellulose film (Seprafilm) might be a valuable new anti adhesion material for abdominal or pelvic surgery and might be superior to existing anti adhesion materials and techniques.

The more viscous solution have a greater ability to separate the injured surfaces and appear to be more slowly absorbed, thereby reducing adhesion formations. Similar benefit was described by Kitano *et al*[17] in 1991. They demonstrated that epidural scar formation was reduced when viscous carboxymethylcellulose solution were instilled after laminectomy in a rabbit model.

Previous studies have shown N,O-carboxymethyl chitosan (NOCC) to be effective in reduction of postoperative adhesions[6,12]. NOCC reduced experimental peritoneal adhesion formation in the rat uterine horn and small bowel laceration models[12]. They hypothesized that NOCC form a barrier to the activation of the normal pathways of fibrin deposition, and this prevent adhesions but do not affect normal wound healing. But the mechanism of action of NOCC is still unclear.

Krause *et al*[20] demonstrated that NOCC, a derivative of chitin that markedly reduced adhesions, suppressed the levels of an inhibitor of cell proliferation released into serum and peritoneal exudates after cecal abrasion in the rat. The action of suppression of transforming growth factor-beta 1 (TGF-beta) reduced adhesion formation. Thus at least one potential effect of NOCC involves a mechanism distinct from TGF-beta inhibition.

Although this researches also could not clear the exact mechanism of anti adhesion of CT, 3% CT significantly reduced the incidence and intensity of postoperative adhesions in the small bowel abrasion model ($p < 0.001$). The results also showed that 3% CT does not adversely affect normal wound healing and healthy recovery after operation.

In summary, it might be concluded that CT was effective on reducing adhesion formation in experimental rat models. But, this results were hard to compare because different animal models, different methods of inducing adhesions, and different routes of administration and dosages were used. Given the large number of experimental studies in several animals, future studies should focus on the clinical use of Chitosan-Trimer in the prevention of postoperative adhesion formation.

References

1. Anup R, Apama V, Pulimood A, Balasubramanian KA.

Surgical stress and the small intestine: Role of oxygen free radicals. Surgery 1999; 125: 560-569.
2. Becker JM, Dayton MT, Fazio VW. Prevention of postoperative abdominal by a sodium hyaluronate-based bioresorbable membrane. J Am Coll Surg 1996; 183: 297-306.
3. Brecht M, Mayer U, Schlosser E, Prehm P. Increased hyaluronate synthesis is required for fibroblast detachment and mitosis. Biochem J 1986; 239: 445-450.
4. Buckman RF, Woods M, Sargent L, Gervin AS. A unifying pathogenetic mechanism in the etiology of intraperitoneal adhesions. J Surg Res 1976; 20: 1-5.
5. Burns JW, Burgess L, Skinner K, Rose R, Colt MJ, Diamond MP. A hyaluronate based gel for the prevention of postsurgical adhesions: evaluation in two animal species. Fertil Steril 1996; 66: 814-821.
6. Costain DJ, Kennedy R, Ciona C, McAlister VC, Lee TDG. Prevention of postoperative adhesions with N,O-carboxymethyl chitosan: examination of the most efficacious preparation and the effect of N,O-carboxymethyl chitosan on postsurgical healing. Surgery 1997; 121: 314-319.
7. Crowe DT, Bjorling DE. Peritoneum and peritoneal cavity. In: Textbook of small animal surgery, 2nd ed, Saunders, Philadelphia. 1993: 407-430.
8. Ellis H. The cause and prevention of postoperative intraperitoneal adhesions. Surg Gyne & Obstet 1971; 133: 497-511.
9. Haney AF, Doty E. A barrier composed of chemical cross-linked hyaluronic acid (Incert) reduces postoperative adhesion formation. Fertil Steil 1998; 70: 145-151.
10. Hellebrekers BWJ, Trimbos-Kemper TCM, Trimbos JBMZ, Emeis JJ, Kooistra T. Use of fibrinolytic agents in the prevention of postoperative adhesion formation. Ferti Steril 2000; 74: 203-212.
11. Hooker GD, Taylor BM, Driman DK. Prevention of adhesion formation with use of sodium hyaluronate-based bioresorbable membrane in a rat model of ventral hernia repair with polypropylene mesh-a randomized, controlled study. Surgery 1999; 125: 211-216.
12. Jenkins SD, Klamer TW, Parteka JJ, Condon RE. A comparison of prosthetic materials used to repair abdominal wall defects. Surgery 1983; 94: 392-398.
13. Johns DS, Kiorpes TC, Rodgers KE, DiZerega GS, Donahue WD. Reduction of adhesion formation by postoperative administration of ionically cross-linked hyaluronic acid. Fertil Steril 1997; 68; 37-42.
14. Kang TY, Choi MC, Lee HJ. Study on adhesion formation by artificial injuries in rats. Korean J Vet Clin Med 1992; 9: 223-228.
15. Kennedy R, Costain DJ, McAlister VC, Lee TDG. Prevention of experimental postoperative peritoneal adhesions by N,O-carboxymethyl chitosan. Surgery 1996; 120: 866-870.
16. King SR, Hickerson WL, Proctor KG. Beneficial actions of exogenous hyaluronic acid on wound healing. Surgery 1991; 109: 76-84.
17. Kitano T, Zerwekh JE, Edwards ML, UsuiYand Allen MD. Viscous carboxymethylcellulose in the prevention of epidural scar formation. Spine 1991; 16: 820-823.
18. Klokkevold PR, Vandemark L, Kenney EB, Bernard GW. Osteogenesis enhanced by chitosan (poly-N-acetyl glucosaminoglycan) in vitro. J Periodontol 1996; 67: 1170-1175.
19. Kocak I, Unlu C, Akcan Y, Yakin K. Reduction of adhesion formation with cross-linked hyaluronic acid after peritoneal surgery in rats. Fertil Steril 1999; 72: 873-878.
20. Krause TJ, Goldsmith NK, Ebner S, Zazanis GA, Mckinnon RD. An inhibitor of cell proliferation associated with adhesion formation is suppressed by N,O-carboxymethyl chitosan. J Invest Surg 1998; 11: 105-113.
21. Leach RE, Burns JW, Dawe EJ, SmithBarbour MD, Diamond MP. Reduction of postsurgical adhesion formation in the rabbit uterine horn model with use of hyaluronate/carboxymethylcellulose gel. Fertil Steril 1998; 69: 415-418.
22. Liebman SM, Langer JC, Marshall JS, Collins SM. Role of Mast Cells in Peritoneal Adhesion Formation. Am J Surg 1993; 165: 127-130.
23. Milligan DW, Raftery AT. Observations on the pathogenesis of peritoneal adhesions: a light and electron microscopical study. Br J Surg 1974; 61: 274-280.
24. Mitchell JD, Lee R, Hodakowski GT, Neya K, Harringer W, Valeri R, Vlahakes GJ. Prevention of postoperative peritoneal adhesions with a hyaluranic acid coation solution. J Thorac Cardiovasc Surg 1994; 107: 1481-1488.
25. Montz FJ, Fowler JM, Wolff AJ, Lacey SM, Mohler M. The ability of recombinant tissue plasminogen activator to inhibit post-radical pelvic surgery adhesions in the dog model. Am J Obstet Gynecol 1991; 165: 1539-1542.
26. Okamoto Y, Shibazaki K, Minami S, Matsuhashi A, Tanioka S, Shigemasa Y. Evaluation of chitin and chitosan on open wound healing in dogs. J Vet Med Sci 1995; 57: 851-854.
27. Osada H, Takahashi K, Fujii TK, Tsunoda I, Satoh K. The effect of cross-linked hyaluronate hydrogel on the reduction of post-surgical adhesion reformation in rabbits. J Int Med Res 1999; 27: 233-241.
28. Thompson JM, Paterson-Brown S, Harbourne T, Whawell SA, Kalodiki E, Dudley HA. Reduced human peritoneal plasminogen activating activity: possible mechanism of adhesion formation. Br J Surg 1986; 76: 382-384.
29. Urman B, Gomel V. Effect of hyaluronic acid on postoperative intraperitoneal adhesion formation and reformation in the rat model. Fertil Steril 1991; 56: 568-570.
30. Vipond MN, Whawell SA, Thompson JN, Dudley HAF. Peritoneal firinolytic activity and intra-abdominal adhesions. Lancet 1990; 335: 1120-1122.

랫트에서 Chitosan-Trimer가 복강유착에 미치는 영향

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초 록 : 복강 내 유착은 단층편평상피로 구성된 복막의 손상으로 장막표면의 염증반응을 일으켜 창상조직의 혈관투과성이 증가하여 많은 장액성 혈액삼출물이 생산되고, 이 혈액삼출물내의 fibrin이 제거되지 않으면 초기섬유소성 유착이 발생한다. 따라서 유착방지는 섬유소성 부착물의 정상적인 용해를 방해하는 인자들과 관계가 있다. Chitosan은 poly-β(1→4)-D-glucosamine으로 chitin을 탈아세틸화시킨 것으로 복강유착방지에 효과가 있다고 알려진 Hyaluronic acid(HA)와 구조상 유사성을 가지고 있다. 본 연구는 쥐에서 회장에 유착을 유도한 후 PBS (control group), 1% Chitosan Trimer (CT, 1% CT group), 3% CT (3% CT group), chitin (chitin group)을 복강내 주입하여서 10일 뒤에 유착방지효과, 유착발생정도, 조직검사, 혈액상의 변화를 관찰하였다. 총백혈구수, 총적혈구수, PCV, PLT, Total protein은 전군에서 유의적인 변화가 나타나지 않았다. 조직검사상에서 유의적인 차이는 나타나지 않았으나, 3% CT군은 다른 군에 비해 Fibrosis와 염증반응정도에 대한 점수가 낮았다. 혈장섬유소원은 전군에서 수술 후 증가하였으나 3% CT군은 대조군에 비해 증가율이 낮아서 유의적인 차이를 보였다 ($p<0.05$). 유착장소는 전군에서 장막-장막 (60%), 장막-장간막 (13.3%), 장막-고환쪽 결합조직 (10%), 대망-간 (10%), 장막-대망 (3.3%), 장막-맹장 (3.3%)순으로 발생하였다. 유착발생빈도는 3% CT군이 62.2%로 대조군 97.7%, 1% CT군 81.8%, chitin군 93.3%에 비해 유의적으로 낮았다 ($p<0.05$). 유착 형성은 대조군, 1% CT, 3% CT 및 chitin 투여군에서 각각 2.07 ± 0.81, 1.03 ± 0.63, 0.64 ± 0.53 및 1.67 ± 0.71로 3% CT군은 대조군에 비해 유의적인 감소를 보였다 ($p<0.001$).