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Effect of carboxymethyl chitosan on postoperative intraperitoneal adhesion formation in the rat

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Abstract : The aim of this study was to determine the effectiveness of carboxymethyl chitosan(CMC) solution on preventing intraperitoneal adhesions. In this study, 44 rats were divided into four groups ; an untreated control group and three experimental groups that were treated with 3 ml of 1, 2 or 3% CMC solution, respectively. 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 0.5 × 1 cm area. The adhesions were blindly assessed 2 weeks later by using a computerized tensiometer. The mean tensile strength(Newton) of formed adhesions was 2.48 ± 0.88 in control group, 1.86 ± 0.56 in the 1% CMC-treated group, 1.75 ± 0.71 in the 2% CMC-treated group and 1.55 ± 0.60 in the 3% CMC-treated group. The most favorable prevention against adhesion was achieved in the 3% CMC-treated group($p<0.01$). We could conclude that CMC was effective on preventing the formation of postoperative small intestinal adhesions in the rat.

Key words : carboxymethyl chitosan, intraperitoneal adhesion, rat, tensile strength.

Introduction

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. A large volume of published research has been devoted to this problem and a substantial number of pharmaceutical agents have been investigated for their ability to prevent adhesions[1~3]. Numerous solutions and techniques have been used in attempts to prevent the formation of intraperitoneal adhesions following surgical trauma to serosal or peritoneal surfaces. Crystalloid solutions, dextran[4~6], corticosteroids[7,8], heparin[9], nonsteroidal anti-inflammatory agents[10], calcium channel blockers[11], progesterone[12], tissue plasminogen activator[13], and several bar-

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rier methods such as amniotic membrane grafts[14], polytetrafluoroethylene surgical membrane[15], and oxidized regenerated celluose[16] are some of the examples that can be mentioned. There has been a lack of corroboration of the studies reporting beneficial results. Because of the limited success obtained with the numerous substances that have been tried, the search for an effective adjuvant to prevent postoperative adhesions has been continued. Earlier animal studies have shown carboxymethyl chitosan(CMC) to be efficacious on the prevention of intraperitoneal adhesions. Chitosan is a useful derivative of chitin. It is formed through N-deacetylation of the chitin molecule. Carboxymethylation is performed so as to make chitosan water soluble, negatively charged, biocompatible polymer that is hydrophilic, lubricious, and viscoelastic. CMC which is a long chain polysaccharide is a novel agent with structural similarities to hyaluronic acid. The most distinctive property of CMC is to retain moisture, and unlike chitosan, they can be made soluble in a wide range of pH levels. CMC is nontoxic, either *in vitro* in fibroblast culture assays or *in vivo* in experimentation with intraperitoneal, oral, or subcutaneous treatment at concentrations higher than those described in this experimentation [17,18]. The aim of this study was to assess the effectiveness of different concentrations of CMC on preventing postoperative abdominal adhesions in rats.

Materials and Methods

Experimental animals : 44 adult female Sprague-Dawley rats weighing 230 to 250 g were used in this experiment. All rats were housed three/cage at 18℃, and artificial light was on from 6 a.m to 6 p.m. They were fed on pellet chow and tap water *ad libitum* before and after surgery. Rats were divided into four groups ; a control group that was untreated and three experimental groups receiving 3 ml of 1, 2 or 3% CMC solution immediately before peritoneal closure, respectively.

Carboxymethyl chitosan(CMC) : The modified CMC solutions were obtained from food grade powder(RC Biochemical Co., Ltd, Pusan, Republic of Korea). Three solutions were prepared that contained 1% CMC, 2% CMC or 3% CMC. All solutions were prepared in sterile, pyrogen-free distilled water, and sterilized by autoclaving for 20 minutes. CMC was administered as a sterile solution to spread evenly over the entire peritoneal cavity before closure.

Surgical protocol : The rats were anesthetized with intraperitoneal thiopental sodium(50 mg/kg). Their ventral hair was shaved with electric clipper, and their abdominal skin was scrubbed with povidone-iodine and rinsed with 70% alcohol. By use of aseptic technique a 5 cm midline skin incision was made and the skin was retracted bilaterally. A 3 cm midline incision was made, and the ileum was then exteriorized. The anti-mesenteric serosa of the ileum approximately 3 cm from the ileocecal junction was then abraded in a standard manner by scraping with a scalpel blade so that a homogenous surface of petechial hemorrhages was created over a 0.5 × 1 cm area. The right abdominal wall was retracted. A 0.5 × 1 cm segment of right parietal peritoneum was sharply excised from the wall including a superficial layer of underlying muscles, 1 cm lateral to the midline incision. Both peritoneal and ileal defects were exposed to air until hemorrhage was disappeared. After air drying, the peritoneal defect and abraded ileal defect were apposed by two interrupted sutures at the cranial and caudal edges with 6-0 polypropylene(Prolene®, Ethicon, UK) to maintain their position. The peritoneomuscle was closed by a simple continuous pattern with 4-0 chromic catgut. Closure of the skin incision was achieved with a simple interrupt suture of 4-0 nylon. In treatment groups, all solutions were instilled into the abdominal cavity through a catheter immediately before closure of the peritoneomuscular layer.

Postoperative evaluation : Two weeks later, euthanasia was performed with halothane and then a postmortem examination was conducted immediately. The segment of the peritoneum and ileum containing the adhesive site was excised to test. The tensile strength of the adhesion site was evaluated with tensiometer(H500DM, Hounsfield Co., UK) in each group(Fig 1). Both the ileal and peritoneal 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

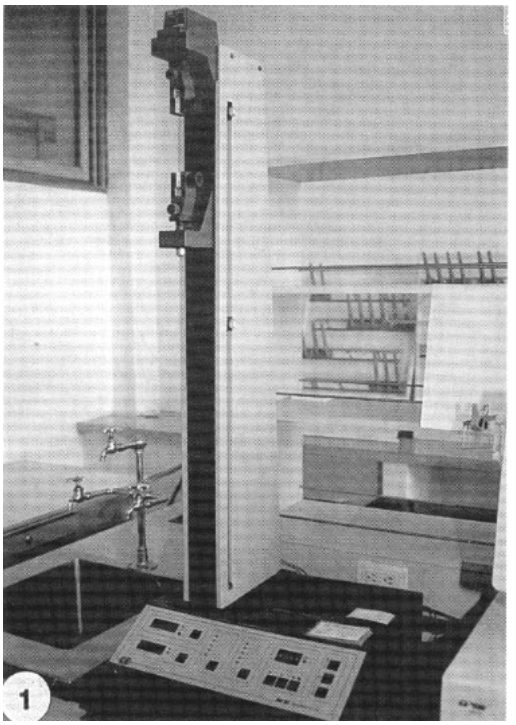

Fig 1. The tensiometer used in the measurement of the force required to separate adhesions.

performed at the room temperature of 20℃, and specimens were moistened with a very fine mist of normal saline while clamped.

For microscopic evaluation, tissue samples were collected from 4 rats in all groups. Collected tissues included adhesions, serosal scars and grossly normal part. Each was fixed in 10% formalin, and multiple segments containing injured area from each group were routinely processed for histologic examination. Sections were stained with hematoxylin and eosin.

Statistics : Statistical analysis of tensile strength was performed in each group using ANOVA. The value considered to be statistically significant was $p<0.01$.

Results

Two animals died unexpectedly on postoperative day 5 and 7 due to complications of peritonitis. These animals, one in control group and the other in 1% CMC treated group, were eliminated from the analysis because their peritonitis was too severe to measure a tensile strength of fibrous adhesions. Surviving animals showed no evidence of other complications. Macroscopic examination of the abdomen after euthanasia revealed no residual viscous solution, adhesion in the abdominal incision site and evidence of ascites. This experimental model resulted in fibrous adhesions in all of control and treatment groups.

The mean tensile strengths of formed adhesions were 2.59 $\pm$ 0.85 newtons in the control group, 1.86 $\pm$ 0.56 newtons in the 1% CMC-treated group, 1.75 $\pm$ 0.71 newtons in the 2% CMC-treated group, and 1.55 $\pm$ 0.60 newtons in the 3% CMC-treated group(Fig 2). High variability was seen in the tensile strength of adhesions in the control and CMC-treated groups. The tensile strengths of the CMC-treated groups, however, were reduced in comparison with that of the control group. The most favorable prevention of postoperative intraperitoneal adhesion was achieved in the 3% CMC-treated group($p<0.01$).

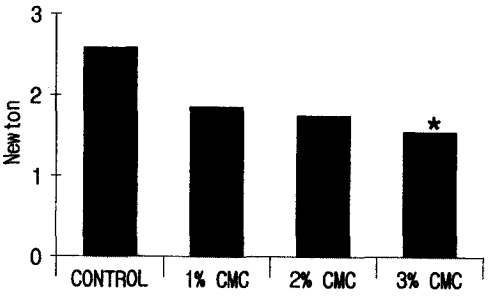

Fig 2. The tensile strength of the adhesion site was evaluated with tensiometer 14 days after surgical procedure in rats.
★ : $p<0.01$ compared with the control group.

No obvious microscopic differences were noted among tissue sections obtained from all groups. In all sections, the following lesions generally were observed ; erosion of areas of muscularis, muscularis and serosal fibroplasia with neovascularization and inflammation, and foreign body granulomas(Fig 3-6).

Discussion

Peritoneal trauma, resulting from such diverse effects as drying, ischemia, thermal injury, infection, or the presence of a foreign body, has long been recognized as a stimulus for adhesion formation[19]. Because of difficulties in elimination of the trauma during surgical manipulation, recent efforts to lower the incidence of adhesion formation have been focused on inhibiting the body's response to these stimuli. The development of adhesions involves a complex interaction between the deposition of the fibrinous exudate and the lysis of the fibrin via the fibrinolytic system of the mesothelial cells[20]. Prevention of adhesion has been performed by the inhibition of some processes in inflammatory response, by the mechanical separation of the visceral surfaces, or by the dilution of the exudates containing fibrin. An ideal antiadhesive agent would coat the wound surface until the surface matured to the point that was no longer susceptible to adhesion formation. Hopefully, the presence of the antiadhesive agent would not interfere with the maturation process taking place at the wound surface. Harris *et al*[20] reported that the fluid such as lactated ringer solution had a rapid absorption rate in the peritoneal cavity with most being absorbed within the first 24 hours of instillation. Because an effective barrier must be present for at least 36 hours after injury in the rat model, single instillation of the solution like saline or lactated ringers is considered to be an ineffective barrier on prevention of adhesion formation because of its rapid absorption. Nonabsorbable barriers were effective on preventing adhesions because they formed a permanent barrier among the injured surfaces. The permanent barriers, however, may not be ideal antiadhesive agents because they remain as permanent foreign bodies in the peritoneal cavity. Coating the abdominal surfaces with viscous solutions to inhibit adhesive contact of injured tissues has met with moderate success. This approach appears to be convenient and uncomplicated if the correct solutions are identified[20]. CMC is a novel agent with structural similarities to hyaluronic acid(HA). HA has been shown to play a role in normal wound healing[21,22] and in inhibiting pericardial[23] and peritoneal[24,25] adhesion formation. HA has proved unattractive in a clinical use because of its high cost and limited efficacy[26,27]. Kennedy *et al*[17] demonstrated that wound healing at the site of both intestinal anastomosis and skin wound is not deleteriously affected by treatment with CMC, and additional studies are warranted to futher define its efficacy, safety, and mechanism of action. Intraperitoneal instillation of CMC solution is frequently used clinically to prevent postoperative adhesion formation despite conflicting results in relation to its efficacy. The explanation for the adhesion prophylaxis properties of CMC solution is unclear, but a "hydroflotation" effect and/or a "siliconizing" effect has been referred to.

This study showed that the 3% CMC solution significantly decreased the tensile strength required to rupture the adhesion site. It is therefore reasonable that our results are consistent with previous studies in rat. Intraabdominal instillation of solutions of carboxymethyl cellulose(0.9% to 3%) has been shown previously to significantly reduce the incidence of postoperative adhesions in rats[28], rabbits[3], ponies[29], and sheep[30]. The effectiveness of the carboxymethyl cellulose solutions appears to be increased with increasing concentrations[3,28,31]. Similarly, the tensile strength in our experiment was decreased with increasing concentrations. The more viscous solutions have a greater ability to separate the injured surfaces and appear to be more slowly absorbed, thereby increasing their residence time on injured surfaces. Similar benefit was obtained in a rabbit model where epidural scar formation was reduced after laminectomy when viscous carboxymethyl cellulose solutions were instilled at the surgical site[32].

Although intraperitoneal instillation of CMC doesn't decreased the extent of adhesion, it significantly decreased the median tensile strength in 1%, 2% or 3%($p<0.01$) CMC-treated group. We could conclude that CMC was effective on preventing the formation of postoperative small intestinal adhesions in the rat. Futher studies are necessary on the model presented herein, other models using different types of CMC and different visceral injury.

Legend for figures

Fig 3. The section from specimen shows a focal mucosal erosion with granulation tissue formations. Variable inflammatory cells and neovascular structures are found in the granulation tissue. Adhesion site of peritoneum shows several foreign body granulomas with impacted silk bundles. This area shows diffuse fibroblastic proliferations with reparative fibrous tissue formations. Serosal area and surrounding fat tissue show mild inflammatory cell infiltrations. Peritoneal mesothelial cells are nonspecific.

Fig 4. The section from specimen shows no mucosal erosion. But, adhesion site reactions are similar to Fig 3. In this specimen, fibroblastic proliferations are inflitrate into deep layer of peritoneal muscle fibers.

Fig 5. The section from specimen shows no mucosal erosion and no granulation tissue formations. Reparative fibrous tissue formations are slightly decreased than Fig 3 & 4.

Fig 6. The section from specimen shows similar findings of Fig 3, 4 and 5. But peritoneal inflammatory changes are more pronounced rather than Fig 3, 4 and 5.

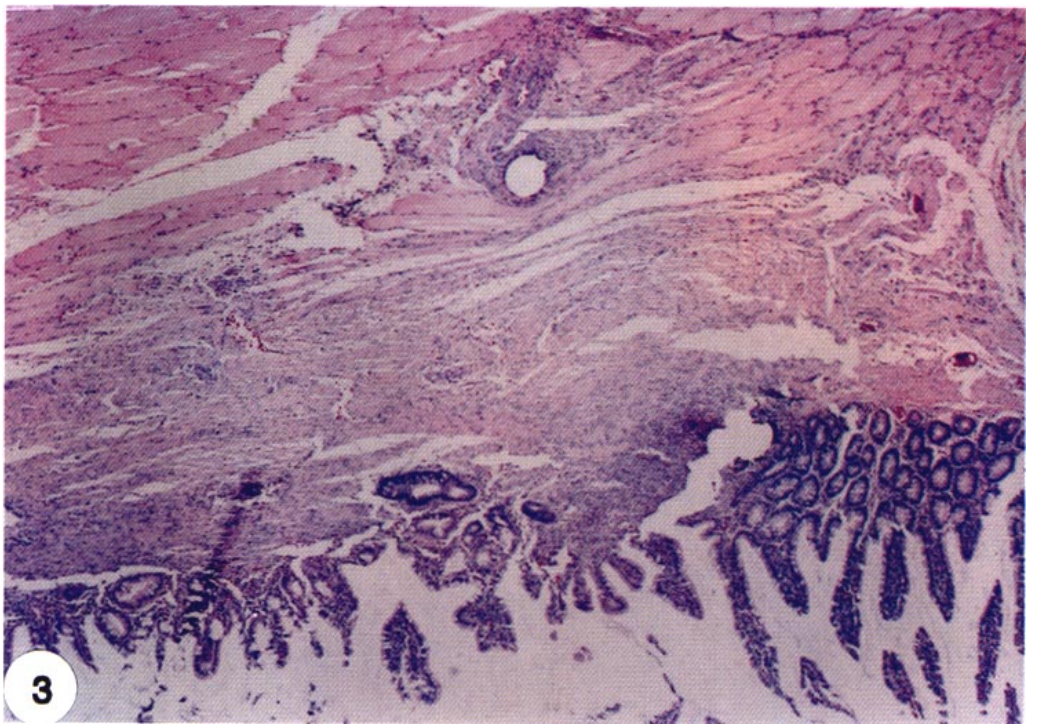

Fig 3.

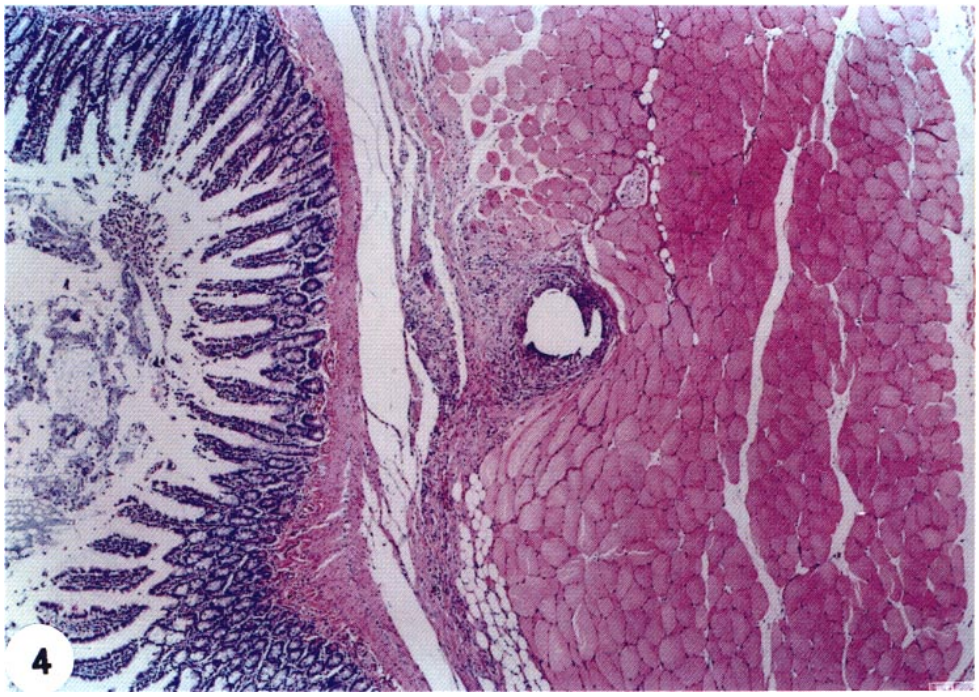

Fig 4.

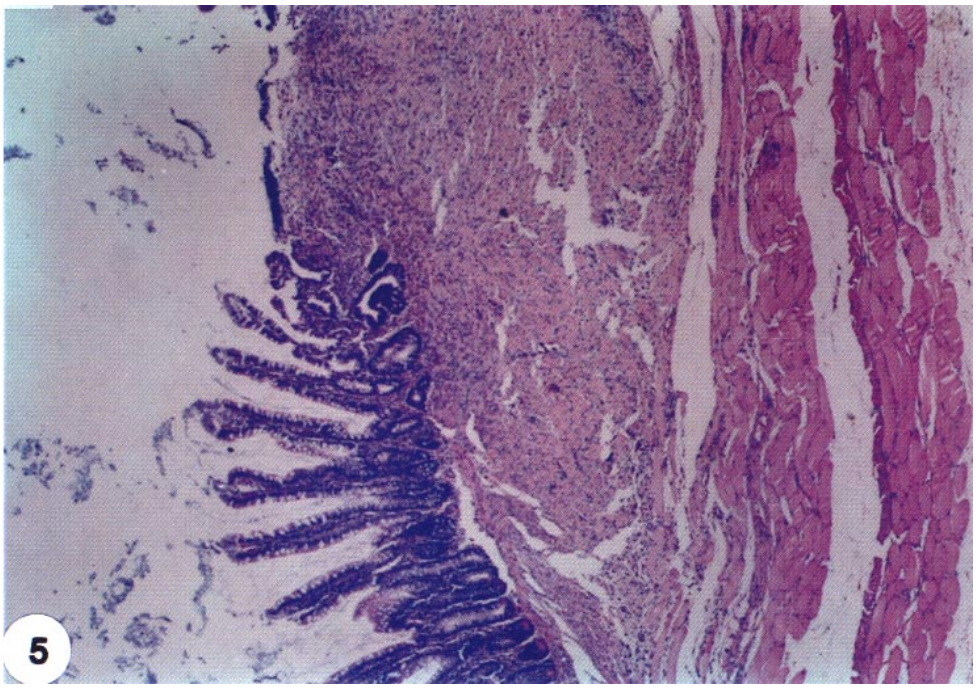

Fig 5.

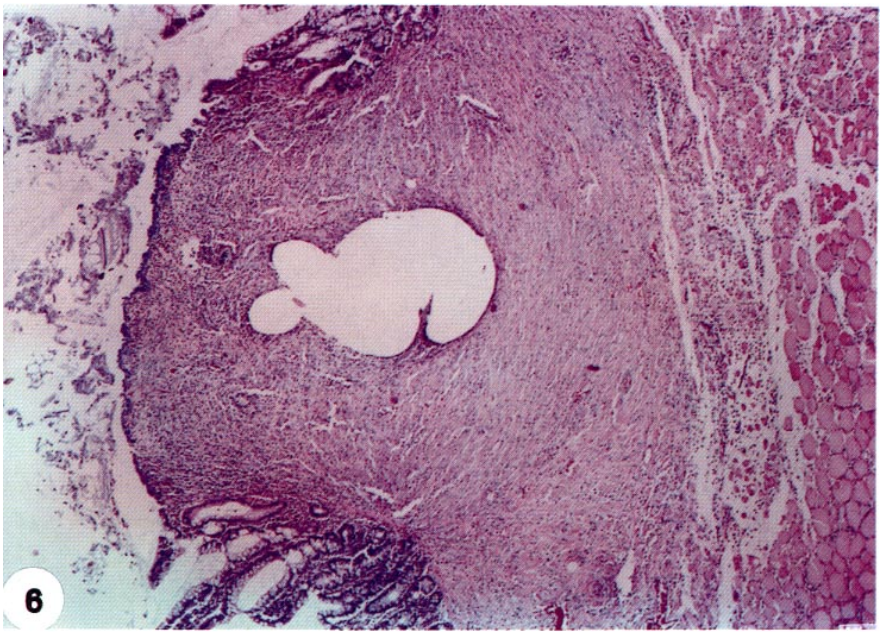

Fig 6.

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Rat에서 carboxymethyl chitosan의 복강수술후 유착형성에 미치는 효과

장광호 · 권영삼 · 김정은 · 권은주 · 오태호 · 이근우 · 장인호

경북대학교 수의과대학

(2000년 6월 10일 게재승인)

국문초록 : 본 실험은 carboxymethyl chitosan(CMC)이 복강유착에 미치는 효과를 밝히고자 수행하였다. 총 44마리의 rat를 유착유도후 아무런 처치하지 않은 대조군과 각각 1, 2, 3% CMC 용액 3ml로 처치한 3개 실험군 등 총 4군으로 나누었다. 유착은 회장의 장간막 반대측 장막부위를 노출시켜 수술도로 0.5×1cm 크기로 긁어 점상출혈을 유도하고, 우측 벽측복막을 0.5×1cm 간격으로 창상을 유도한 후 두 창상을 봉합하였다. 2주후 halothane에 의한 안락사 후 유착정도를 computerized tensiometer로 측정하였다. 유착부 분리에 필요한 장력(Newton)은 대조군이 평균 2.48±0.88이었으며, 1% CMC 처치군은 1.86±0.56, 2% CMC 처치군은 1.75±0.71, 3% CMC 처치군은 평균 1.55±0.60로 3% CMC 처치군에서 유의적인 감소($p<0.01$)가 나타났다. 이상의 결과로 미루어 rat에서 복강수술후 CMC의 복강내 투여는 복강장기 유착방지에 효과적이라고 사료된다.

Key words : carboxymethyl chitosan, intraperitoneal adhesion, rat, tensile strength.