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RAPID COMMUNICATION

Preventive effect of gelatinizedly-modified chitosan film on peritoneal adhesion of different types

Xie-Lai Zhou, Shan-Wen Chen, Guo-Dong Liao, Zhou-Jun Shen, Zhi-Liang Zhang, Li Sun, Yi-Jun Yu, Qiao-Ling Hu, Xiao-Dong Jin

Xie-Lai Zhou, Shan-Wen Chen, Guo-Dong Liao, Xiao-Dong Jin, Department of Urology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China
Zhou-Jun Shen, Department of Urology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200025, China
Xie-Lai Zhou, Zhi-Liang Zhang, Yi-Jun Yu, Surgical Department, Clinical Medical College of Hangzhou Teachers College, Hangzhou 310036, Zhejiang Province, China
Li Sun, Experimental Center of Medical Science, Hangzhou Teachers College, Hangzhou 310036, Zhejiang Province, China
Qiao-Ling Hu, Institute of Polymer Composites, Zhejiang University, Hangzhou 310027, Zhejiang Province, China
Supported by The National Natural Science Foundation of China, No. 50173023
Correspondence to: Xiao-Dong Jin, Department of Urology, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China. addamm@mail.hz.zj.cn
Telephone: +86-571-87236833 Fax: +86-571-87236628
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Abstract

AIM: To comparatively study the preventive effect of gelatinizedly-modified chitosan film on peritoneal adhesions induced by four different factors in rats.

METHODS: Chitosan was chemically modified by gelatinization, and made into films of 60 μm in thickness, and sterilized. Two hundred Sprague-Dawley rats were randomly divided into five groups, Sham-operation group (group A), wound-induced adhesion group (group B), purified talc-induced adhesion group (group C), vascular ligation-induced adhesion group (group D), and infection-induced adhesion group (group E), respectively. In each group, the rats were treated with different adhesion-inducing methods at the cecum of vermiform processes and then were divided into control and experimental subgroups. Serous membrane surface of vermiform processes were covered with the films in the experimental subgroups, and no films were used in the control subgroups. After 2 and 4 wk of treatments, the abdominal cavities were reopened and the adhesive severity was graded blindly according to Bhatia's method. The cecum of vermiform processes were resected for hydroxyproline (OHP) measurement and pathological examination.

RESULTS: Adhesion severity and OHP level: After 2 and 4 wk of the treatments, in the experimental subgroups, the adhesions were significantly lighter and the OHP levels were significantly lower than those of the control subgroups in group B (2 wk: 0.199 ± 0.026 *vs* 0.285 ± 0.041 μg/mg pr, $P < 0.001$; 4 wk: 0.183 ± 0.034 *vs* 0.276 ± 0.03 μg/mg pr, $P < 0.001$), D (2 wk: 0.216 ± 0.036 *vs* 0.274 ± 0.040 μg/mg pr, $P = 0.004$; 4 wk: 0.211 ± 0.044 *vs* 0.281 ± 0.047 μg/mg pr, $P = 0.003$) and E (2 wk: 0.259 ± 0.039 *vs* 0.371 ± 0.040 μg/mg pr, $P < 0.001$; 4 wk: 0.242 ± 0.045 *vs* 0.355 ± 0.029 μg/mg pr, $P < 0.001$), but there were no significant differences in groups A (2wk: 0.141 ± 0.028 *vs* 0.137 ± 0.026 μg/mg pr, $P = 0.737$; 4 wk: 0.132 ± 0.031 *vs* 0.150 ± 0.035 μg/mg pr, $P = 0.225$) and C (2 wk: 0.395 ± 0.044 *vs* 0.378 ± 0.043 μg/mg pr, $P = 0.387$; 4 wk: 0.370 ± 0.032 *vs* 0.367 ± 0.041 μg/mg pr, $P = 0.853$); Pathological changes: In group B, the main pathological changes were fibroplasias in the treated serous membrane surface and in group D, the fibroplasia was shown in the whole layer of the vermiform processes. In group E, the main pathological changes were acute and chronic suppurative inflammatory reactions. These changes were lighter in the experimental subgroups than those in the control subgroups in the three groups. In group C, the main changes were foreign body giant cell and granuloma reactions and fibroplasias in different degrees, with no apparent differences between the experimental and control subgroups.

CONCLUSION: The gelatinizedly-modified chitosan film is effective on preventing peritoneal adhesions induced by wound, ischemia and infection, but the effect is not apparent in foreign body-induced adhesion.

Key words: Chitosan; Gelatinization; Chemical modification; Peritoneum; Adhesion

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INTRODUCTION

Peritoneal adhesion is a kind of defensive reaction to peritoneal injury. However, it can also result in intestinal obstruction and cause severe clinical disorders. Therefore, it is important to prevent peritoneal adhesion in abdominal surgical operations. Unfortunately up to now, there have been no ideal methods to prevent peritoneal adhesion in clinical practice. Chitosan is a deacetylated derivate from chitin. Many previous studies showed that chitosan has effects of haemostasis, and sterilization, facilitates epithelial reparation and inhibits fibroblastic growth[1-3]. Chitosan has been used to prevent tissue adhesions, such as peritoneal adhesion, tendon adhesion and synarthrophysis[4-6]. In clinical application, it was found that the gel flows easily and is difficult to stay in the target regions for sufficient time, and the gel also degrades so fast that it could only maintain effectiveness for a short duration. In order to delay the degradation and decrease the fluidity of the gel, in our previous study, we processed the chitosan to films and transplanted it into the abdominal cavity of rats. But the film degraded too slowly and 8 wk after the transplantation, most of the films resided in the cavity. The residual film was encapsulated by the surrounding tissue and the peritoneal adhesion was worsened. In order to overcome these disadvantages, we mixed gelatin to chitosan and produced blending films. The blending film degraded much faster than the previous pure chitosan film, but it also produced foreign body reactions and formed severe foreign body granuloma around the blending film[7].

To solve the above problems and develop a useful chitosan film to prevent the peritoneal adhesion, in the present study we chemically modified the chitosan by gelatinization to develop a new sort of chitosan film, and comparatively studied the preventive effect of the film on peritoneal adhesions induced by wound, infection, ischemia and foreign body in rats.

MATERIALS AND METHODS

Animals and grouping

Two hundred Sprague-Dawley rats, one half for each gender, weighing 200-250 g, were provided by the Experimental Animal Center of Zhejiang Province. They were randomly divided into five groups: sham-operation group (group A), wound-induced adhesion group (group B), purified talc-induced adhesion group (group C), vascular ligation-induced adhesion group (group D), and infection-induced adhesion group (group E). Each of the above groups was subdivided into two subgroups, experimental and control subgroups (20 rats for each subgroup and one half for each gender). All the rats were fed under the same conditions: at 24-26℃ of environmental temperature, about 40% humidity, with an alternate 12 h light/dark cycle, and free access to food and water.

Preparation of chitosan film

The chitosan (from Yuhuan Aoxing Chitin Ltd., Zhejiang Province, China) was dissolved, purified, gelatinizedly modified, filtered and made into films. The films were dried and dissected to patches of 10 cm × 10 cm in size and 60 μm in thickness, and the film patches were sterilized by radiation of ^{60}Co for later use.

Surgical operation

Under general anaesthesia with intraperitoneal injection of 3% amobarbital (60-90 mg/kg), the rats were immobilized in dorsal position, routinely degermed, abdominally incised through a median incision of 2-3 cm long, and their vermiform processes were searched and pulled out of the incisions, then the terminal vermiform processes within a length of 3 cm were treated as follows: In group A, the vermiform processes were exposed to air for 5 min; in group B, the anterior surface of serous membrane was scraped slightly with surgical blade till obvious congestion and small bleeding drops appeared; in group C, 10 mg of talc powders were evenly smeared over the anterior surface of serous membrane; in group D, the vermiform artery stem was ligated with $0^{\#}$ surgical thread at a point of 3 cm from the dead end in the following way: loosely knotting the first loop, thrilling a thread with equivalent diameter to the vermiform artery stem through the first loop, tightening the first loop, pulling out the thrilled thread, and knotting and tightening the second loop of the ligation knot. The ligation resulted in a stricture of vermiform artery, which induced ischemia of the distal vermiform tissue from the ligation point. In group E, the dead end of the vermiform process was poked out with a hole using a $16^{\#}$ needle, a drop of intestinal content was extruded out and evenly smeared over the anterior surface of serous membrane, and then the remaining content in the vermiform process was pushed to the cecum. After the above treatments, for the experimental subgroups, the treated serous membranes were covered with the prepared chitosan films, and the vermiform processes were put back into the abdominal cavity, which were then closed. For the control subgroups, all the treatments were the same as those of the experimental subgroups except that the chitosan film was not placed. The duration from opening to closing the abdominal cavity was 5 min, so that the duration of exposure of intestines to air was the same for each rat.

At 2 and 4 wk after the surgery, 10 rats (5 female and 5 male) in each subgroup were randomly selected respectively and their abdominal cavities were reopened under anaesthesia, and the grades of peritoneal adhesion were evaluated, which existed between the treated vermiform processes and intestines, mesenteries and abdominal walls. After that, the vermiform processes with adhesions were resected and washed with normal saline, and then were divided into two segments for each resected process. The proximal segments were fixed with formalin and histopathologically examined, and the distal segments were stored at -80℃ for measurement of hydroxyproline (OHP).

Grading standard for peritoneal adhesion

According to Bhatia's[8] grading method of 5 levels and considering the characteristics of peritoneal adhesion in rats, we formulated the following grading standard:

Table 1 Comparison of peritoneal adhesive grades between experimental and control subgroups within each group

Group	Control group (n = 10)					Experimental group (n = 10)					U	P
	0	Ⅰ	Ⅱ	Ⅲ	Ⅳ	0	Ⅰ	Ⅱ	Ⅲ	Ⅳ		
Group A: sham-operation												
2 wk	9	1	0	0	0	10	0	0	0	0	45.000	0.317
4 wk	10	0	0	0	0	9	1	0	0	0	45.000	0.317
Group B: wound-induced adhesion												
2 wk	0	5	4	1	0	6	3	1	0	0	14.500	0.005
4 wk	0	5	5	0	0	7	3	0	0	0	7.500	0.001
Group C: purified talc-induced adhesion												
2 wk	0	0	1	3	6	0	0	1	2	7	45.500	0.687
4 wk	0	0	1	4	5	0	0	0	4	6	43.000	0.547
Group D: vascular ligation-induced adhesion												
2 wk	0	4	4	2	0	3	6	1	0	0	18.000	0.009
4 wk	1	4	4	1	0	5	4	1	0	0	21.500	0.023
Group E: infection-induced adhesion												
2 wk	0	0	1	4	5	0	4	5	1	0	5.500	0.001
4 wk	0	0	1	5	4	0	6	4	0	0	2.000	< 0.001

Grade 0: no adhesions; Grade Ⅰ: the ratio of adhesive area/total treated area in the vermiform processes is < 50%, and the adhesion is easily to be dissected bluntly; Grade Ⅱ: the ratio is ≥ 50%, and the adhesion is easily to be dissected bluntly; Grade Ⅲ: area of the adhesion is out of consideration. Although blunt dissection for the adhesion can be carried out, it is difficult and the intestinal wall will be impaired after the blunt dissection; Grade Ⅳ: area of the adhesion is out of consideration. The adhesion is fast and cannot be bluntly dissected. Each rat was graded by three referees blindly and the average grade of the three was accepted as the adhesive grade of the rat.

Determination of total protein and OHP

The levels of total protein and OHP in the adhesive tissue were determined using the corresponding kits supplied by Nanjing Jiancheng Bioengineering Institute, China. The determining processes completely followed the instructions of the kits. Contents of OHP in the adhesive tissue were calculated as micrograms of OHP in each milligram of total protein (μg/mg pr).

Statistical analysis

All the data were processed with SPSS10.0. Mann-Whitney U test of non-parametric statistics for independent samples was used to analyze differences in the peritoneal adhesive grades and t-test was used to analyze differences in OHP levels between the experimental and control subgroups within each group.

RESULTS

Gross findings

Abdominal incisions healed in first grade in all rats of all groups. There was no obvious postoperative abdominal infection in groups A, B, C and D at 2 and 4 wk after the surgical operations. There was no residual chitosan film in the abdominal cavities of rats in all experimental subgroups.

Comparison of peritoneal adhesion grade

As it shows in Table 1, within group A (sham-operation group) and group C (talc-induced adhesion group), there was no significant difference in peritoneal adhesion between the experimental and control subgroups both at 2 and 4 wk ($P > 0.05$). Within group B (wound-induced adhesion group), group D (vascular ligation-induced adhesion group) and group E (infection-induced adhesion group), the peritoneal adhesion grades of experimental subgroups were significantly lower than those of corresponding control subgroups ($P < 0.05$) both at 2 and 4 wk after the surgical treatments. It indicates that the gelatinizedly modified chitosan film has remarkable effect on preventing peritoneal adhesions induced by wound, ischemia and infection, but no obvious effect on adhesion induced by talc powders. From the results in Table 1, we also concluded that in group E, the mean decreased adhesion grades were 1.7 and 1.4 from experimental to control group at 2 and 4 wk respectively. While in group B, the mean decreased grades were 1.1 and 1.2, and in group D, the mean decreased grades were 1.0 and 0.9. It suggests that the modified chitosan film is more effective on preventing infection-induced peritoneal adhesion than on wound and ischemia induced adhesion.

Comparison of OHP levels in adhesive tissue

As it shows in Table 2, in groups A and C, there were no significant differences in OHP levels between the experimental and control subgroups both at 2 and 4 wk ($P > 0.05$). In groups B, D and E, the OHP levels of experimental subgroups were significantly lower than those of corresponding control subgroups ($P < 0.05$) both at 2 and 4 wk after the surgical treatments. The changes in OHP levels were concordant with the changes in the adhesive grades, and it was confirmed to have a peritoneal adhesion-preventive effect when the gelatinizedly modified chitosan film was applied to regions with wound, ischemia and infection in abdominal surgical operations.

Comparison of pathological changes

In group A, there were no obvious pathological changes in vermiform processes of all rats. In groups B and D, there were obvious fibroplasias and sporadic infiltration of lymphocytes in serous membrane (group B) and the whole

Table 2 Comparison of OHP levels between experimental and control subgroups within each group (μg/mg pr)

Group	Control group (n = 10)	Experimental group (n = 10)	t	P
Group A: sham-operation				
2 wk	0.137 ± 0.026	0.141 ± 0.028	0.331	0.737
4 wk	0.150 ± 0.035	0.132 ± 0.031	1.217	0.225
Group B: wound-induced adhesion				
2 wk	0.285 ± 0.041	0.199 ± 0.026	5.602	< 0.001
4 wk	0.276 ± 0.038	0.183 ± 0.034	5.768	< 0.001
Group C: purified talc-induced adhesion				
2 wk	0.378 ± 0.043	0.395 ± 0.044	0.874	0.387
4 wk	0.367 ± 0.041	0.370 ± 0.032	0.182	0.853
Group D: vascular ligation-induced adhesion				
2 wk	0.274 ± 0.040	0.216 ± 0.036	3.408	0.004
4 wk	0.281 ± 0.047	0.211 ± 0.044	3.438	0.003
Group E: infection-induced adhesion				
2 wk	0.371 ± 0.040	0.259 ± 0.039	6.34	< 0.001
4 wk	0.355 ± 0.029	0.242 ± 0.045	6.675	< 0.001

layer (group D) of the adhesive vermiform processes at 2 wk, and the main pathological change was fibroplasia at 4 wk after the surgical treatments. The above pathological changes were milder in experimental subgroups than those in control subgroups except for changes in group D and at 4 wk. In group C at 2 wk, there occurred obvious foreign-body giant cell reactions, granuloma, fibroplasias and sporadic infiltration of lymphocytes at the treated serous membranes of adhesive vermiform processes, and the granuloma and fibroplasias became severer at 4 wk. There were no significant differences in the above pathological changes between the experimental and control subgroups. In group E, the main pathological change in the treated region was acute suppurative inflammation at 2 wk, and chronic inflammatory reaction characterized with granulation and fibroplasias at 4 wk after the surgical treatments. The above inflammatory reactions were milder in experimental subgroup than those in control subgroup both at 2 and 4 wk.

DISCUSSION

Chitosan is chemically termed β-(1, 4)-2-amino-2-deoxy-D-dextran, and its main component is glucosamine. Glucosamine is also a sort of internal substance in human bodies, therefore, chitosan is biocompatible. If the chitosan is introduced into animal or human bodies, it will be degraded into small molecules of oligosaccharides and absorbed by the bodies without causing any acute or chronic toxicity. Chitosan is a derivant of deacetylized chitin, and the chitin is the major component of outer shells of invertebrates. Because of these characteristics of chitosan, it is widely and deeply researched in areas of pharmaceutical preparations and medical polymer synthesis[9].

Peritoneal adhesion occurs in more than three fourths of patients following laparotomy. The outcomes of adhesion are unpredictable and diverse, causing a significant health care burden. Intestinal obstruction, infertility, problems at reoperative surgery and cumulative costs of care over many years are the key concerns[10]. The peritoneal adhesion develops only several hours after the abdominal surgical operations. At first, the serous fluid exudes from the injured sides of intestinal wall, and then fibrinogen in the serous fluid transforms to fibrin and coagulates, thereby membranous peritoneal adhesion in the injured intestinal wall is formed. After that, the fibrinolytic system is activated and the fibrin is lyzed, thereby the membranous peritoneal adhesion is gradually eliminated. If the fibrin cannot be totally lyzed, the left fibrin will be organized and develop fibrinous adhesion, which usually forms at 2 wk after the surgical operation[11]. Based on the mechanisms through which the chitosan prevents peritoneal adhesion: inhibiting growth of fibroblasts, facilitating reparation of the epithelium, and disinfection, it is concluded that the chitosan can only prevent against pre-fibrinous adhesion. Once the fibrinous adhesion is formed, chitosan is useless. Therefore, when we use chitosan film to prevent peritoneal adhesion, the optimal duration for the film to stay in the abdominal cavity is within 2 wk. Firstly, the film can exert an effect of mechanical isolation in a solid state before it is completely degraded; On the other hand, when the film is degraded, the released chitosan monomers can also take anti-adhesive effect. In the present study, we gelatinizedly modified the chitosan to develop a new sort of chitosan film, which can be slowly dissolved in water. The experiments demonstrated that, within 2 wk after the film was transplanted into the abdominal cavity of the rats, it was completely degraded and absorbed without any foreign-body granuloma reaction. This suggests that the gelatinizedly modified chitosan film has the potential to be biomaterial for adhesion-prevention.

There are many factors that can induce peritoneal adhesions, of which the main factors are wound, ischemia, infection and foreign bodies. In most cases of clinical peritoneal adhesion, the adhesions are caused by combined factors, among which one or several factors play major roles[11,12]. The present study utilized rat models to investigate effects of the gelatinizedly modified chitosan film on peritoneal adhesions induced by four different factors. The results demonstrated that, at 2 and 4 wk after the surgical operations, the chitosan films significantly reduced the adhesion grades in groups of wound, ischemia and infection-induced adhesions. This suggests that the chitosan films have obvious preventing effects on wound, ischemia and infection-induced peritoneal adhesions. The results also demonstrated that the films are more effective on infection-induced peritoneal adhesion. The mechanism may be as follows: The chitosan film prevents infection-induced peritoneal adhesion not only through promoting the epithelium recovery and inhibiting the growth of fibrous tissue, but also through its anti-infection effect by inhibiting hyperplasia of granulation and fibrous tissue. Through the double pathways the chitosan may inhibit the adhesion more strongly. It also seemed that healing of the abdominal incisions is not obviously affected by the chitosan transplantation. With respect to effects of the chitosan film on intra-abdominal anastomotic stoma healing, it needs to be clarified further.

Within the talc powder-induced adhesion group, there were no significant differences in adhesive grades between the experimental and control subgroups both at 2 and 4 wk. This showed that the gelatinizedly modified chitosan film has no obvious preventive effect on foreign body-induced peritoneal adhesion. The reason is as follows: For the talc powder-induced adhesion, the main pathological changes are foreign-body granuloma complicated with a large quantity of fibroplasias, and these changes will maintain as long as the foreign bodies exist. However, the chitosan film degraded in a relative fast rate in the abdominal cavity and there was no obvious residual film at 2 wk after the transplantation. Therefore, the fast degraded film cannot exert a strong effect on a chronic and persistent foreign-body granuloma reaction.

There is a high content of OHP in collagen protein, a very low content in elastin protein, and none in other sorts of proteins. Ozogul *et al*[13] reported that there existed positive correlation between adhesive grades and OHP levels in the adhesive tissue, and concluded that OHP is a significant index to measure the adhesive degree of tissue, which is more sensitive and objective than the index of gross adhesive grade. In the present study, the OHP changing tendency within each group and differences in OHP levels between subgroups were concordant to those of adhesive grades. This further confirms the preventive effect of gelatinizedly modified chitosan film on wound, ischemia and infection-induced peritoneal adhesions.

COMMENTS

Background

Peritoneal adhesion can cause intestinal obstruction and other severe clinical disorders, so it is very important to prevent peritoneal adhesion in abdominal surgical operations. But up to now, there are still no ideal methods to prevent peritoneal adhesion in clinical practices. Chitosan is a deacetylated derivate from chitin. Many studies reported that chitosan was applied to prevent tissue adhesions, such as peritoneal adhesion, tendon adhesion and synarthrophysis, but the effect was not satisfactory.

Research frontiers

Chitosan is a sort of natural biological material and it has been processed into many forms for medical use. In the area of prevention of peritoneal adhesion with chitosan, the research hotspot is how to modify the chitosan by chemical and physical methods to improve its effectiveness on preventing the adhesion, and simultaneously reduce its adverse reactions.

Innovations and breakthroughs

In the previous application of chitosan gels to the prevention of peritoneal adhesion, it was found that the gel was much fluid and was difficult to stay in the target places for sufficient time, and moreover, the gel degraded so fast that it could only maintain the effectiveness for a short duration. In order to delay the degradation and decrease the fluidity of the gel, we processed pure chitosan into films, but the film degraded too slowly and the residual film was encapsulated by surrounding tissue and the peritoneal adhesion was worsened. In order to overcome these disadvantages, we mixed chitosan with gelatin and produced blending films. The blending film degraded much faster than the previous pure chitosan film, but it also created foreign body reaction and formed severe foreign body granuloma around the blending film. In the present study we chemically modified the chitosan in gelatinization to develop a new sort of chitosan film, and showed that the film is remarkably effective on preventing peritoneal adhesions induced by wound, ischemia and infection except the foreign body-induced adhesion.

Applications

The study results suggest that the gelatinizedly-chitosan film is a potential therapeutic material that could be used in preventing peritoneal adhesions induced by wound, ischemia and infection.

Terminology

Peritoneal adhesion: Peritoneal adhesion is a sort of defensive reaction to the peritoneal injury mainly including wound, infection, ischemia and foreign body, but it can also develop intestinal obstruction and cause severe clinical disorders; chitosan: Chitosan is a deacetylated derivate from chitin, and chitin is the second most abundant natural biopolymer derived from exoskeletons of crustaceans and also from cell walls of fungi and insects.

Peer review

This is a good descriptive study in which authors analyze the preventive effect of gelatinizedly-modified chitosan on peritoneal adhesions induced by different factors in rats. The results are interesting and suggest that gelatinizedly-chitosan is a potential therapeutic substance that could be used in preventing peritoneal adhesions induced by wound, ischemia and infection.

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