Original Article

Effect of Poly lactic-co-glycolic acid-Ibuprofen on ICAM-1 and VCAM-1 Expression in a Mice Adhesion Model

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Abstract

Background: In this study, we compared the effect of ibuprofen (IB) while incorporating by Poly Lactic-co-Glycolic Acid (PLGA) nanofiber on expression of adhesion molecules ICAM-1 and VCAM-1 in a mice adhesion model.

Materials and Methods: Using an adhesion model were induced in mice, PLGA-IB and PLGA membranes and IB were sutured between the abdominal wall and peritoneum after surgical operation to reveal the best membrane for prevention of postoperative adhesion bands by comparison of ICAM-1 and VCAM-1 expression.

Results: Compared with other groups, PLGA-IB showed a greater ability to reduce ICAM-1 and VCAM-1 expression.

Conclusion: These results suggested that in considering the FDA approved polymers, PLGA-IB could be introduced as a potential candidate for prevention of abdominal post-surgery inflammation and adhesion band formation after surgeries.

Keywords: Intraperitoneal adhesion, PLGA, ibuprofen, ICAM-1, VCAM-1

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Introduction

Peritoneal adhesions are a frequent problem that forms following surgery, trauma and infection. Peritoneal adhesion bands are the consequence of inflammatory reactions after damage to the peritoneum[1,2]. Series of inflammatory responses come after injury of the peritoneum that consisting release of white blood cells and platelets, fluid exudation, hyperemia[3,4].

During inflammation, activated T cells producing pro-inflammatory cytokines induce the expression of adhesion molecules (VCAM-1, ICAM-1) which allow for the recruitment of immune cells responsible for

tissue damage[5]. The incidence of adhesion bands formation after surgery is very high. Adhesion bands can cause severe pain, bowel obstruction and infertility. Consequently, adhesion prevention is very important for scientists[6,7]. For this goal, several classes of pharmacologic agents have been tested and used to prevent or reduce adhesions in patients. For instance, anti-adhesive absorbable and nonabsorbable agents such as antibiotics, anti-inflammatory drugs, fibrinolytic agents, and solid barriers as well as some natural materials such as amnion and collagen could have some successes to prevent postsurgical adhesions[8-10].

Electrospun membranes have attracted the interest of research because of their bulk semi conductivity[11]. For the first time Zong has shown that these membranes are effective in prevention of formation of adhesion band[12]. Dinarvand et al., investigated the anti-adhesive and anti-inflammatory effects of polycaprolactone (PCL), poly-L-lactide (PLLA), poly lactic-co-glycolic acid (PLGA), and polyethersulfune (PES) in comparison with the oxidized-regenerated cellulose (Interceed). Their finding revealed that PLGA showed a greater ability to reduce adhesions[8]. According to adhesion bands are induced by inflammatory by inflammatory responses following surgery, we hypothesized that ibuprofen as an anti-inflammatory drug whereas incorporated with PLGA nanofiber might prevent intra-abdominal adhesion formation along with decreasing inflammation at the site of injury.

The present study was designed to compare anti-adhesion potential of IB-incorporated PLGA with PLGA and IB alone in mice adhesion model and examine the expression of VCAM-1 and ICAM-1.

Methods

Electrospun Nanofiber Fabrication: A PLGA (5 wt%) solution was prepared by dissolving PLGA in chloroform/DMF (3/1 v/v) under constant stirring for 2 hours at room temperature. For preparation of the PLGA-IB, IB with a weight ratio of 10% w/w referred to the PLGA were dissolved in 2 ml chloroform and then added to a 4 wt% PLGA solution under constant stirring for 4 hours to obtain a uniform solution. IB loaded nanofiber meshes were obtained by one-step two-nozzle electrospinning of PLGA-IB and PLGA solutions.

The electrospinning experimental setup was a nano model (Tehran, Iran) with two nozzles. During electrospinning, positive 20 and 18 kV charges were applied at the tip of the syringe needle for PLGA-IB and PLGA, respectively. The PLGA and PLGA-IB mass flow rates were maintained at 0.5 and 0.4 ml/h, and the operating distances were selected at 20 and 15cm, respectively. A rotary aluminum foil collector with 300 rpm was used. It was mentioned, Application of a voltage between the needle and the collector forced the solution droplets to leave the needle and spread on a cylinder in the form of ultrafine fibers[13,14].

Experimental Design: In the present study, the surgical procedures for this investigation were performed in accordance with the Stem Cell Technology Research Center (Tehran, Iran) guidelines. A total of 28 male NMRI mice (Razi Institute, Karaj, Iran) were randomly divided into four groups of seven mice as follows: group 1, surgical abrasion without any treatment (n=7); group 2, surgical abrasion plus IB (n=7); group 3, surgical abrasion plus PLGA membrane (n=7); group 4, surgical abrasion plus PLGA-IB membrane (n=7). The animals were kept in 21±2°C and 12/12 hours light/dark conditions. The surgical treatment was performed under general anesthesia induced by ether and ketamine (3 mg/kg) in combination.

Surgical Technique: In this study, the adhesion induction model described by Hemadeh et al., (1993) was used, which resulted in a 100% incidence of adhesions in our control mice. Briefly, following anesthetic induction, animals were placed in a supine position for shaving and sterilization with alcohol and povidine-iodine. A vertical midline incision of two cm. was made in the skin and the abdomen was opened. The exposed cecum was then gently abraded using dry gauze pads at all surfaces until it lost its shine, and hemorrhagic points became visible without perforation. After that, the cecum was returned to its anatomic position in the abdominal cavity. Before closing the abdomen, 1.5×1.5 cm piece of each type of membrane were sutured between the abdominal wall and peritoneum. The abdominal wall was sutured in two layers. After one week, mice were euthanized and their abdominal cavities reopened.

Table 1: Primers used in Real-Time RT-PCR (ICAM-1, VCAM-1 and Beta actin).

Gene	Forward	Reverse	Product size(bp)
ICAM-1	CTTTGAGAACTGTGGCACC	TGAGGTCCTTGCCTACTTG	119
VCAM-1	CAGGTGGAGGTCTACTCATTC	AGGGATACACATTAGGGACTG	109
Beta actin	CTTCTTGGGTATGGAATCCTG	GTGTTGGCATAGAGGTCTTTAC	95

RNA Isolation: The animals in 4 groups (PLGA-IB/PLGA/IB/control) were humanely killed 1 week after the surgery, and the cecum was immediately dissected. The adhesion tissue was placed immediately into RNA later (Qiagen) for preservation and stored at -20°C. Thawed tissues were homogenized in 1 ml of TRIzol reagent (Invitrogen, Carlsbad, CA), and total RNA in the tissue was isolated. Synthesis of cDNA was performed with M-MuLV reverse transcriptase and random hexamer, according to the manufacturer's instructions (Fermentas).

Real time PCR: Primers used for qPCR reactions are detailed in table 1. PCR amplification was performed using Maxima™SYBR Green/Fluorescein qPCR Master Mix (Fermentas) and Beta-actin transcripts served as endogenous controls. All reactions were performed in triplicates. Changes in mRNA expressions were normalized to the relevant internal control, and subsequently calculated using the $2^{-\Delta\Delta Ct}$ method. The Rotor gene 3000 detection system (Corbett) was used for quantitative mRNA transcript expressions.

Statistical analysis: Relative gene expression levels of different genes were analysed by ANOVA using REST software (2009, QIAGEN, Valencia, USA). Data are presented as mean±SEM and $P<0.05$ was considered to be significant.

Results

Macroscopic Examination: No mortalities were observed during or end of the study. Significantly highest *decrease* of adhesion score observed in animals were implanted with PLGA-IB, although treated animals with PLGA have also causes to decrease of adhesion score in comparison to untreated control groups . In addition, adhesion score of animals treated with alone ibuprofen not significantly changed in comparison with control groups. Gross photograph of grade abdominal adhesion also presented in figure 1.

Gene Expression Analysis: Expression of mRNA was analyzed with regard to control group. In this study, expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) has been evaluated. The data for gene expression analysis, comparison to control group revealed that the expression of two studied genes increased in IB group and decreased in PLGA-IB and PLGA groups. Furthermore, in PLGA-IB group, the expression of ICAM-1 and VCAM-1 decreased remarkably (Figure 2).

Discussion

Postoperative adhesions are a significant health problem that occurs after abdominal surgery. This problem increases the cost of health care after surgery. Therefore many scientists have studied for reducing and prevention of these bands[15,16].

Recently, several biomaterials introduced as physical barrier for this goal. For instance Bölgen et al., was incorporated an antibiotic in poly (ε-caprolactone) (PCL) and investigate the effect of this membrane on prevention of adhesion bands[17]. Dinarvand et al., shown that poly lactic-co-glycolic acid (PLGA) have better effect for solving this problem[8].

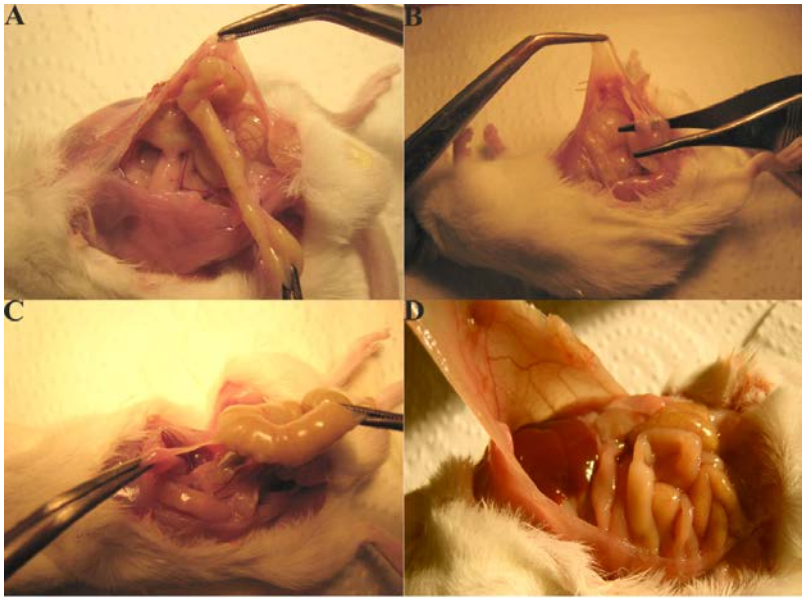

Figure 1. Images show the intraperitoneal adhesion formation in mice. (A) Severe adhesions (control), (B) moderate adhesions (IB), (C) Mild adhesions (PLGA), (D) Without adhesions (PLGA-IB)

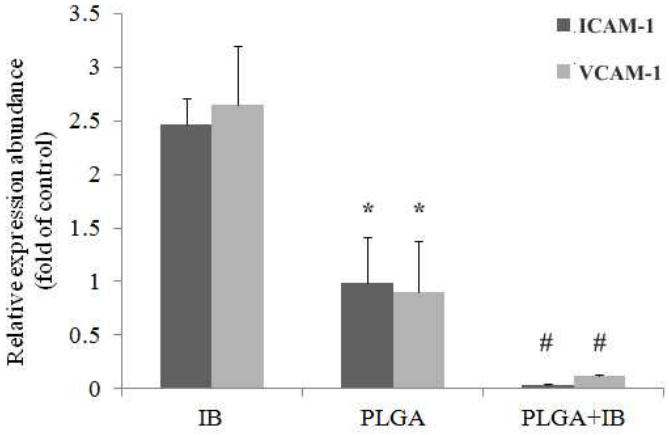

Figure 2. Gene expression analysis .Adhesion molecules genes including ICAM-1 and VCAM-1 were analysed by Real Time-PCR. * indicates a significant level of relative gene expression of PLGA compared to IB group at P value≤0.05; # indicates a significant level of gene expression in PLGA-IB compared to PLGA group at P value≤0.05.

Post-operative inflammation also contributes to surgical adhesion and should be avoided[18]. In this study we used ibuprofen as an anti-inflammatory drug whereas incorporated with PLGA and we examined the preventive effect of PLGA-IB in comparison to PLGA and IB group alone.

VCAM-1 and ICAM-1 expression levels in all groups were important in this study. Our results demonstrated that ICAM-I was down regulated in PLGA-IB group in comparison to control group by a mean factor of 0.031 and VCAM-1 was down regulated by a mean factor of 0.119. The gene expression analysis have shown that there was a significant level of relative gene expression of PLGA compared to IB group and a significant level of gene expression in PLGA-IB compared to PLGA group. The studies have displayed that during inflammation, expression of VCAM-1 and ICAM-1 is induced as well as inflammation is blocked by inhibition of VCAM-1[19,20]. In this project we have seen there was

much inflammation in control an IB groups, but in PLGA-IB there was no trace of inflammation. In PLGA group inflammation has determined. The gene expression data showed that there was a meaningful relation between inflammation and adhesion molecules expression.

Conclusion

In this study, we have shown the effect of PLGA-IB membrane in the prevention of adhesion band that happened after surgery. Indeed, in our work we showed the inhibitory potential of PLGA-IB in the creation of adhesions following surgery. It would be appealing to evaluate a membrane for future therapy.

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Conflict of Interests

The authors declare that they have no competing interests.

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Ethical Approval

All animal care and procedures were approved by the ethical committee of Tehran University of Medical Sciences.

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