The Anti-Adhesive Effect of Granulocyte-Colony Stimulating Factor on an Abdominal Adhesion Model in Sprague-Dawley Rats

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Abstract

Background: Intra-abdominal adhesions are among the most common complications following abdominal surgery. In this study, using an abdominal adhesion rat model, we investigated the anti-adhesive effect of granulocyte-colony stimulating factor (G-CSF) on intra-abdominal adhesions.

Methods: All rats were laparotomized with 3 ischemic peritoneal buttons developed to cause adhesions. The experimental rats were divided randomly into 3 groups (n=8/group): control, pluronic gel, and G-CSF groups. Fourteen days after surgery, all rats were sacrificed, and intra-abdominal adhesions were assessed. The percentage of adhesions, adhesion severity scale and density of adhesion formation were evaluated. Real-time PCR was conducted to assess the cytokine mRNA levels of substance P (SP), neurokinin 1 receptor (NK-1R), transforming growth factor-β1 (TGF-β1), and intercellular adhesion molecule-1 (ICAM-1).

Results: The severity scores of intra-abdominal adhesions of, and the degree of adhesion formation in, rats treated with G-CSF were significantly lower than those in case of rats from other groups. Additionally, in the G-CSF group, the number of ischemic buttons with developed adhesions was significantly lower than that in the other groups. In adhesion samples of the G-CSF group, the expression level of SP was significantly lower than that in the other groups.

Conclusions: Our study demonstrated that G-CSF treatment decreases the formation of intra-abdominal adhesion after surgery by reducing inflammatory reactions in adhesion tissues.

Background

Adhesions, which are described as an irregular union of two opposing tissue surface, are a common result of the normal healing reaction. After abdominal surgery, most patients develop intraperitoneal adhesions [1, 2]. Intra-abdominal adhesions are an important cause of postoperative morbidity. These adhesions are known to be complications associated with abdominal surgery, and they can cause abdominal pain, bowel obstruction, infertility, and chronic pelvic pain [3].

Inflammation is the initial reaction to abdominal trauma during adhesion formation, and it plays an important part in the formation of intra-abdominal adhesions [4]. Fibrin exudation and deposition triggers the development of intra-abdominal adhesions due to an inflammatory reaction in the damaged peritoneum. Moreover, a reduction in the capability of lysis fibrin in damaged sites can also lead to adhesion [5].

Granulocyte colony-stimulating factor (G-CSF) is a cytokine known for stimulating the mobilization of hematopoietic cells from the bone marrow to the peripheral blood. One function of G-CSF is to stimulate the proliferation and differentiation of neutrophil precursors [6]. Recent studies have shown that G-CSF modulates the expression of specific anti-inflammatory cytokines. These experiments revealed that G-CSF decreases the levels of pro-inflammatory cytokines by inhibiting the development of inflammatory

mediators, including interferon-γ, tumor necrosis factor-α, and interleukin-1 [7, 8]. In addition, our previous study reported that the wound healing effect of the local G-CSF injection accelerated the deposition of collagen within the dermis around the wound, resulting in a decreased inflammatory response in wound tissues [9].

Based on reports regarding inflammation, we speculated that G-CSF may have anti-adhesive effects in adhesion formation. We investigated the anti-adhesive effect of G-CSF in a rat model of intra-abdominal adhesion and explored the expression of various inflammatory cytokines in adhesion tissues.

Methods

Animals

In this study, 6-week-old male Sprague-Dawley (SD) rats purchased from Koatech Inc., Republic of Korea, weighing 260–280 g were used. The SD rats were kept at the Animal Experiment Center of Hanyang University in a specific pathogen-free environment. Temperatures were kept at 23–25℃ and humidity was maintained at 50–60% with an artificial light/dark cycle of 12:12 h. The animal research protocol was approved by the Institutional Animal Care and Use Committee of Hanyang University (HY-IACUC-20-0003), and animal studies were conducted in accordance with the Animals in Research: Reporting In Vivo Experiments (ARRIVE) guidelines [10].

Establishment of the abdominal adhesion model

The abdominal adhesion model was induced in rats using procedures based on previously described methods [11]. The rats anesthetized with a combination of rompun (10 mg/kg, Bayer, Seoul, Korea) and zoletil 50 (30 mg/kg, Virbac SA, Carros, France). All rats underwent ventrotomy through a 3 cm skin incision in the midline of the abdomen. Three ischemic buttons were prepared within the parietal peritoneum by gripping a 5 mm parietal peritoneum button with a mosquito hemostat and connecting the bottom of the segment with a 4-O black silk suture (AILEE Co., Pusan, Korea) as described previously [12]. The rats were divided randomly into three groups. Rats in the control group received no medication (n = 8). Rats in the pluronic gel group (n = 8) received only Pluronic F127 gel on the ischemic buttons, and rats in the G-CSF group (n = 8) received a mixture of Pluronic F127 gel and G-CSF (60 μg, Dong-A, Seoul, Korea) on the ischemic buttons (Fig. 1A). The incision was closed with a 3-O polyglycolic acid suture (AILEE Co.) for the fascia and a continuous 5-O polyglycolic acid suture (AILEE Co.) for the skin. After two weeks, the euthanasia of rats induced by carbon dioxide (CO_2) inhalation to obtain samples for molecular analysis and to evaluate and score the adhesion.

Scoring for adhesion

At 2 weeks after surgery, all rats were assessed according to the standard adhesion scoring system [12–14]. The percentage of adhesions was calculated as the number of adhesions in a rat. The adhesion severity scale was characterized as follows: grade 0 = no adhesions, grade 1 = avascular or thin adhesion, grade 2 = dense or vascularized adhesion, and grade 3 = firm or cohesive attachment. The adhesion density was characterized as follows: grade 0 = no adhesions, grade 1 = adhesion was isolated from tissue without gentle traction, grade 2 = adhesion was isolated with mild traction, and grade 3 = adhesion required dissection as described previously [15]. All rats were evaluated by two observers who were blinded to the animal groups.

RNA isolation and real-time PCR

RNA of the adhesion tissues was extracted by QiAzol Lysis reagent (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. A Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) was used to test the concentration of each RNA sample. RNA was then reverse-transcribed with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For quantitative real-time polymerase chain reaction (qRT-PCR) analysis, the LightCycler 480 SYBR Green I Master Mix (Roche Diagnostics, Indianapolis, IN, USA) and LightCycler 480 program (Roche) were used. The genes selected were substance P (SP), neurokinin 1 receptor (NK-1R), transforming growth factor-β1 (TGF-β1), and intracellular adhesion molecule-1 (ICAM-1). Then, qRT-PCR was performed using the LightCycler 480 program (Roche), under the following condition: amplification for 10 min at 95℃; 45 cycles of 95 ℃, 60 ℃, 72 ℃ for 10 s each; and dissociation for 15 s at 65 ℃. The crossing point (CP) value was calculated by the LightCycler 480 program (Roche), and the relative rate of change was calculated using the mRNA ratio of the target gene to that of glyceraldehyde-3-phosphate dehydrogenase.

Statistical analyses

Statistical analyses were conducted using the Statistical Package for the Social Sciences 24.0 software (IBM Co., Armonk, NY, USA). All data are expressed as the mean ± standard error (SE). The statistical difference between groups was analyzed using one-way analysis of variance for multiple comparisons, and post hoc multiple comparisons were performed with Tukey's test (equal variances assumed). P-values less than 0.05 were considered statistically significant.

Results

Development of the abdominal adhesion model

The ischemic buttons were generated on the parietal peritoneum on day 0 (Fig. 1). Adhesion in the G-CSF group was confirmed on day 14 after treatment with G-CSF and pluronic gel. The arrows indicate the ischemic buttons with attached adhesions (Fig. 2).

Adhesions severity scale

The adhesion severity scale was assessed by the adhesion scoring system. The severity of adhesions was significantly lower in the group of rats treated with G-CSF than that in the untreated group (1.19 ± 0.21% vs. 2.52 ± 0.11%, $p < 0.05$). In addition, the adhesion severity was significantly lower in G-CSF-treated rats than in treated with pluronic gel (1.19 ± 0.21% vs. 1.96 ± 0.13%, $p < 0.05$) (Fig. 3A).

Grade of adhesion density

The grade of adhesion density was assessed by the adhesion scoring system. This grade was significantly lower in the group of rats treated with G-CSF than in the untreated rats (0.95 ± 0.18% vs. 2.43 ± 0.14%, $p < 0.05$). In addition, the grade of adhesions density was significantly lower in treated with G-CSF than in those treated with pluronic gel (0.95 ± 0.18% vs. 2.0 ± 0.22%, $p < 0.05$) (Fig. 3B).

Percentage of ischemic buttons with adhesion formation

The percentage of adhesion formation was significantly lower in the rats treated with G-CSF than in the untreated rats (66.67 ± 12.60% vs. 100.0%, $p < 0.05$). In addition, the percentage of adhesion formation was significantly lower in the rats treated with G-CSF than in the rats treated with pluronic gel (66.67 ± 12.60% vs. 95.24 ± 4.76%, $p < 0.05$) (Fig. 3C).

Molecular analysis

The expression level of SP was significantly lower in adhesion samples from rats treated with G-CSF than in those from untreated rats or rat treated with pluronic gel (0.08 ± 0.02% vs. 0.14 ± 0.03% and 0.16 ± 0.03%, respectively; $p < 0.05$ for both comparisons). The expression levels of NK-1R, TGF-β1, and ICAM-1 were not significantly different among the groups (Fig. 4).

Discussion

In this study, we examined the anti-adhesive effect of G-CSF in an abdominal adhesion rat model, as well as the expression of several inflammatory cytokines in adhesion tissues. Our study showed that G-CSF treatment decreased the severity of adhesions, grade of the adhesion density, and the percentage of adhesion formation in the abdominal adhesion rat model. Additionally, G-CSF treatment reduced SP mRNA expression in the adhesion tissues.

Intra-abdominal adhesion formation following surgery can result in serious complications, including abdominal pain, intestinal obstruction, and possible chronic pelvic pain [3, 16]. Various surgical methods have been used to treat abdominal adhesions; adjuvants have also been proposed for treating adhesion formation. The development of surgical techniques may reduce abdominal adhesion, but such

techniques cannot eliminate it completely [17–19]. Many anti-adhesion agents are used, which can be classified as systemic or intraperitoneal agents and intraperitoneal barriers [2, 20, 21]. However, despite these anti-adhesion agents, many problems arise. The adhesion formation process has been explored in different ways and can be divided into four stages: an inflammatory post-injury stage, a fibrin dissolving stage, a fibrous band stage, and a phagocytic and remodeling stage [22–24]. Many studies have determined that inflammatory responses play an important role in the formation of adhesions [25, 26]. *Wei et al*. [27] reported that gallic acid reduces adhesions formation in rats by inhibiting inflammatory reactions. Our findings also showed that the inflammatory cytokine level of SP was decreased by G-CSF treatment. Moreover, adhesion formation has also been found to be reduced, along with a reduction in the inflammatory response. Therefore, the inflammatory response may play a significant role in the development of adhesions.

SP is secreted by a variety of inflammatory cells, including lymphocytes, eosinophils, macrophages, and dendritic cells. SP also functions by binding to the NK-1 receptor [28]. Some studies have shown that preventing the pro-inflammatory effects of SP may have potential therapeutic effects in inflammation related diseases [29, 30]. Our study confirmed that G-CSF treatment decreases SP mRNA expression in adhesion tissues, which indicated that SP indirectly prevents adhesion formation.

G-CSF is an important regulator of hematopoiesis and the innate immune system; for example, it stimulates the proliferation, survival and differentiation of neutrophil precursors and mature neutrophils and the mobilization of bone marrow cells [31]. However, recent studies shown that G-CSF exerts a local effect on the healing process in damaged tissues [32]. In particular, our previous study showed that the local effect of G-CSF reduces the inflammatory response during the healing process, as well as accelerates this process [9]. Our results also showed that the local actions of G-CSF reduced abdominal adhesions and inflammatory cytokines.

This study has several limitations. First, the multiple mechanisms underlying the anti-adhesive effects of G-CSF on adhesion formation could not be clearly defined. Second, we did not set an optimal dosage range for G-CSF. Further investigations concerning the appropriate dose of G-CSF are thus required. Finally, although we found that G-CSF reduced abdominal adhesion, we did not compare the results with those of other anti-adhesion agents. Further studies will need be required to compare the effects of G-CSF against those of other anti-adhesion agents.

Conclusion

In conclusion, our research showed that G-CSF treatment reduced the development of postoperative intra-abdominal adhesions and SP mRNA expression in adhesion tissues. Anti-inflammatory activity is also a possible mechanism of underlying the action of G-CSF. The anti-adhesive effect of G-CSF is associated with the alleviation of inflammatory reactions, which may play an important role clinically.

Abbreviations

Neurokinin 1 receptor: NK-1R, transforming growth factor-β1: TGF-β1, Intercellular Adhesion Molecule 1: (ICAM-1), glyceraldehyde-3-phosphate dehydrogenase: GAPDH, crossing point: CP, specific pathogen-free: SPF, interleukin-1: IL-1, tumor necrosis factor-α: TNF- α, interferon gamma: IFN-γ, and granulocyte-colony stimulating factor: G-CSF

Declarations

Ethics and Consent to participate

The protocol of animal research animal research protocol was accepted by the Institutional Animal Care and Use Committee of Hanyang University (HY-IACUC-20-0003), and animal studies were conducted in accordance with the Animals in Study Guidelines Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Consent for publication

Not applicable.

Availability of data and material

The datasets used are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

I.H.P, Y.S.S., K.S.K contributed to conception or design. I.H.P, G.Y.S, N.K.S, A.H.L. contributed to acquisition, analysis, or interpretation of data. Y.S.S, H.W.J, H.K, K.S.K. contributed to drafting the work or revising. All authors read and approved the final manuscript.

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Figures

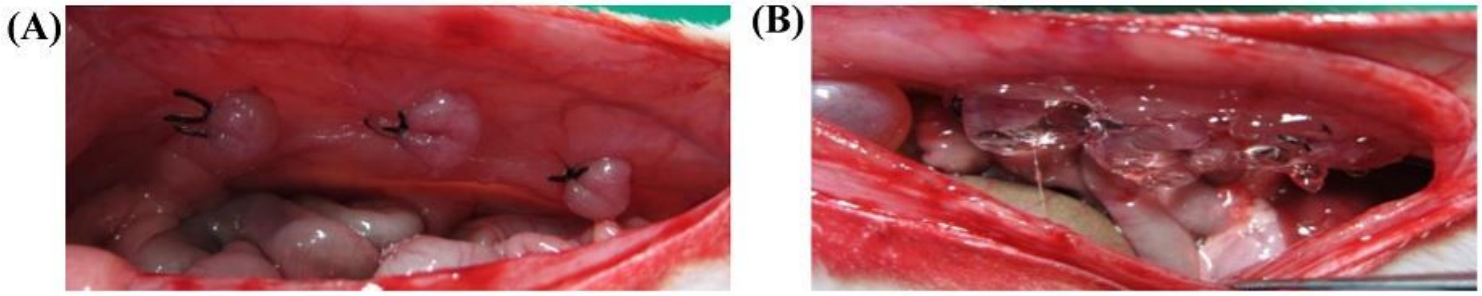

Figure 1

Creation of the ischemic buttons. (A) The ischemic buttons on the parietal peritoneum were created by gripping a 5 mm parietal peritoneum button with a mosquito hemostat and ligating the bottom of the segment. (B) Treatment of the ischemic buttons with G-CSF and pluronic gel.

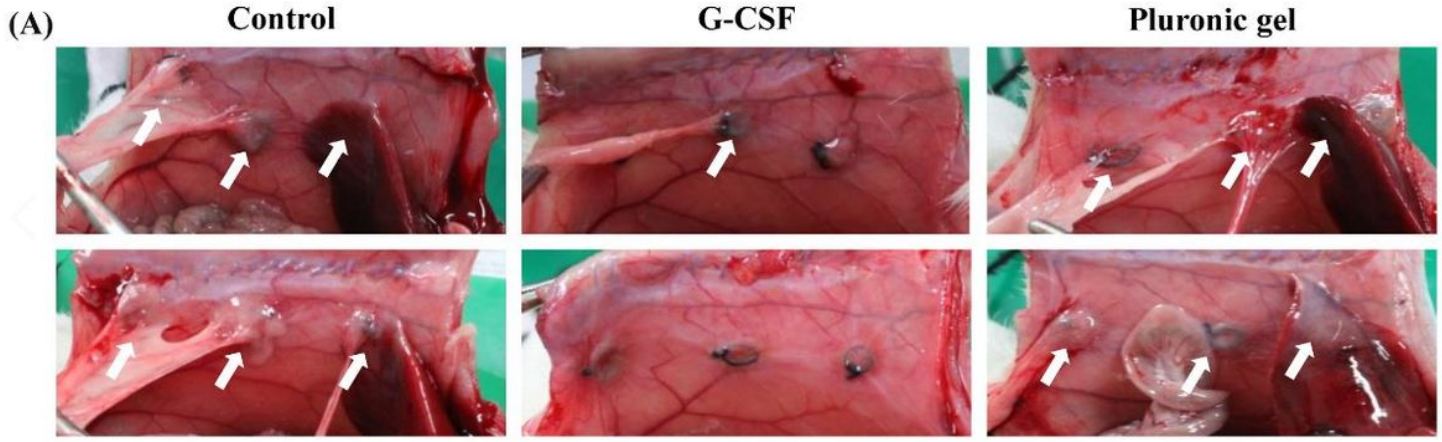

Figure 2

Abdominal adhesions after 2 weeks. The white arrows indicate the ischemic buttons with attached adhesions.

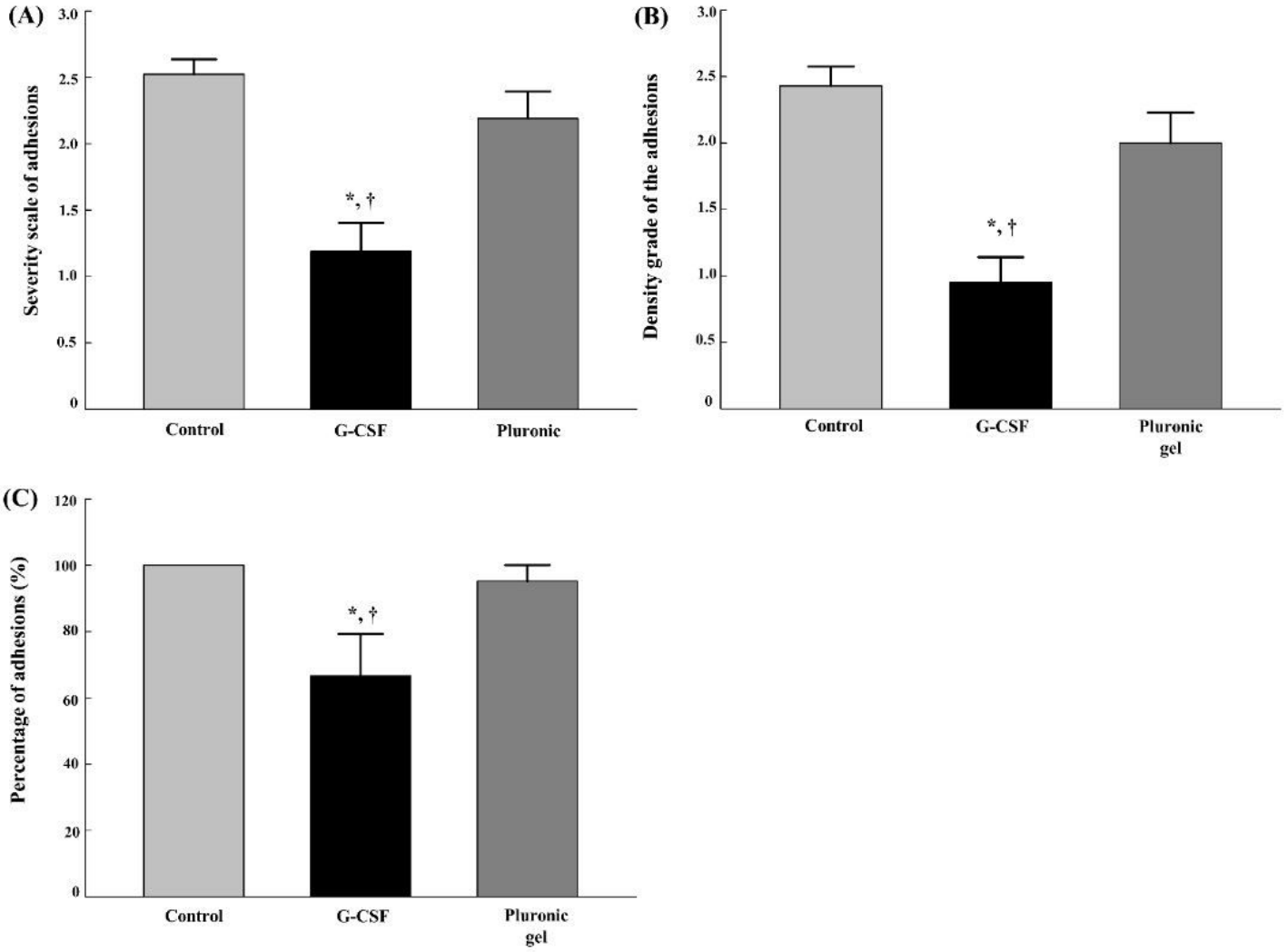

Figure 3

Assessment of adhesion scores in each group. (A) Scale of adhesion severity in each group at 2 weeks after treatment. (B) Grade of adhesion density in each group at 2 weeks after treatment. (C) Percentage of ischemic buttons with adhesion formation in each group at 2 weeks after treatment. All data are expressed as the mean ± standard error (n=8 per group). *P < 0.05 vs. control, †P < 0.05 vs. pluronic gel.

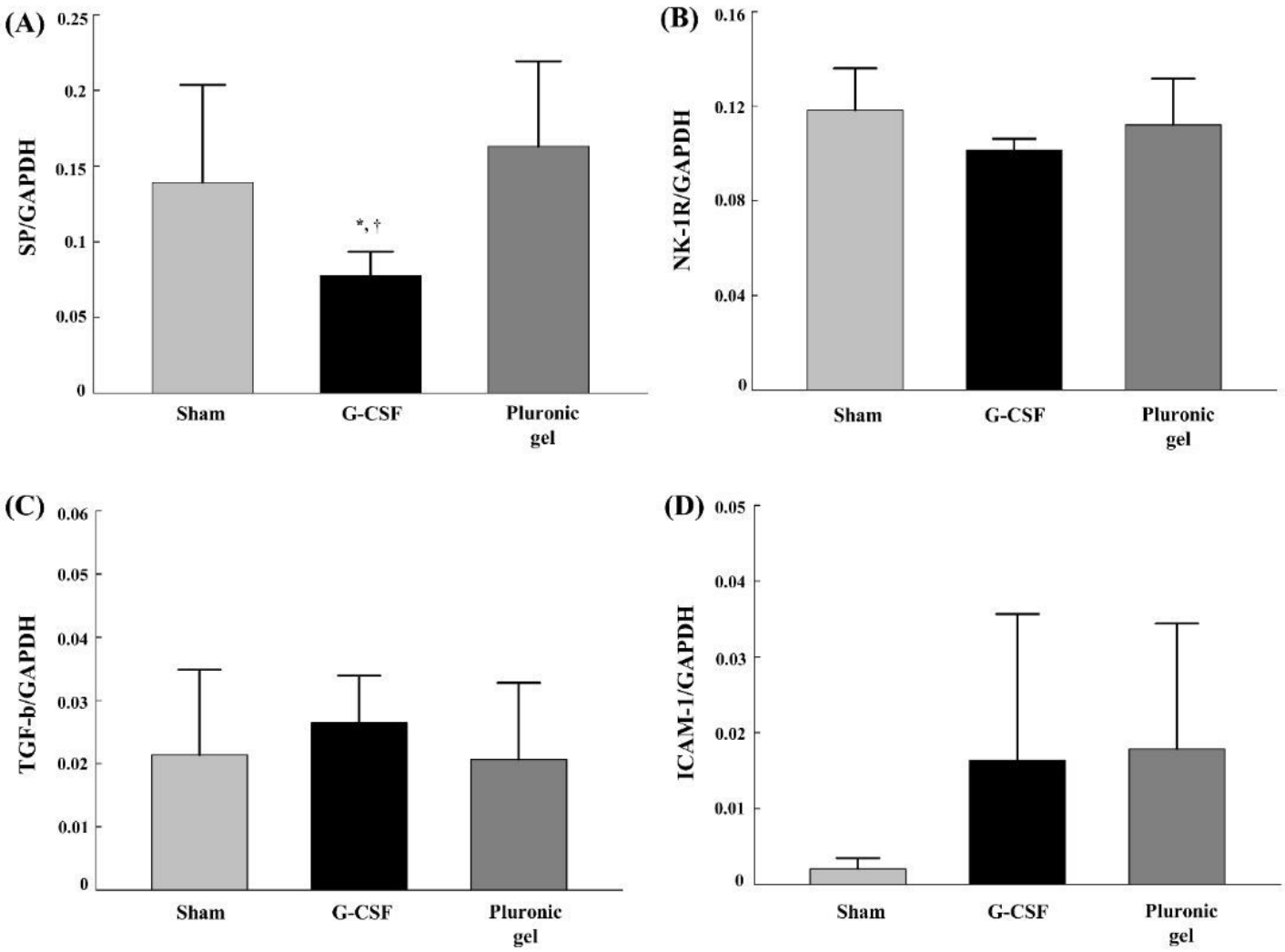

Figure 4

The relative mRNA expression levels of SP, NK-1R, TGF-β1, and ICAM-1 in peritoneal adhesion tissues from rats with ischemic buttons at 2 weeks after treatment. All data are expressed as the mean ± standard error (n=8 per group). *P < 0.05 vs. control, †P < 0.05 vs. pluronic gel. SP, Substance P; NK-1R, neurokinin 1 receptor; TGF- β1, transforming growth factor-β1; and ICAM-1, Intercellular adhesion molecule-1.

Supplementary Files

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