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Intestine

Inhibition of JAK1 mitigates postoperative ileus in mice

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ABSTRACT

Background: Intestinal inflammation is the predominant contributor to the genesis of postoperative ileus. Janus kinase 1 plays an important role during inflammation. Here, we investigated the role of Janus kinase 1 in postoperative ileus and whether inhibition of Janus kinase 1 could mitigate postoperative ileus.

Methods: A mouse model of postoperative ileus was induced by intestinal manipulation. Janus kinase 1 inhibitor GLPG0634 or placebo was administered orally before intestinal manipulation. At the indicated time points post operation, neutrophil infiltration was assessed by immunohistochemistry and enzyme-linked immunosorbent assay; proinflammatory gene expression was quantified by quantitative reverse-transcriptase polymerase chain reaction and enzyme-linked immunosorbent assay; and Janus kinase 1 activation was detected by Western blot. Functional studies were conducted to evaluate intestinal motility.

Results: We found that intestinal manipulation led to marked activation of Janus kinase 1, with increased proinflammatory gene expression and upregulated myeloperoxidase level. Moreover, intestinal manipulation resulted in an impairment of intestinal transit in vivo and inhibition of smooth muscle contractility in vitro. Preoperative administration of GLPG0634 markedly lowered the expression of proinflammatory cytokines, the myeloperoxidase level in the muscularis layer after bowel manipulation, and significantly ameliorated smooth muscle contractile function and intestinal transit ability.

Conclusion: Our data showed that Janus kinase 1 activation mediated intestinal manipulation-induced resident macrophage activation after intestinal manipulation, and subsequent complex inflammatory cascade and gut dysmotility. Janus kinase 1 inhibition appears to be a prospective and convenient approach for the prevention of postoperative ileus.

Introduction

Postoperative ileus (POI), a frequent surgical complication, could lead to postoperative morbidity and prolonged hospitalization, thereby causing enormous socioeconomic burden.[1] Moreover, there are few effective methods for POI prophylaxis or treatment.[2,3] Since recent studies have elucidated that local muscle inflammatory response triggered by intestinal manipulation (IM) during surgery significantly contributed to the development of POI,[4–7] research on prevention and treatment of POI should focus on inhibiting the muscular inflammation.

Resident macrophage activation within the muscularis externa has been demonstrated to be an initial driver of the severe postoperative inflammation resulting in POI. Wehner et al[8] found that resident macrophages are activated early after IM, resulting in subsequent inflammatory cascade and intestinal dysfunction. Furthermore, they reported that inhibition of the P38 signal pathway using the macrophage-specific inhibitor semapimod prevented intestinal macrophage activation and completely abrogated smooth muscle dysfunction caused by IM.[9] These findings suggest that inhibiting macrophage activation by signal pathways may be an effective strategy for POI prevention.

Considering that signal pathways may be involved in regulating IM-induced macrophage activation and inflammation, a candidate is Janus kinase 1 (JAK1). JAK1 is a member of the Janus kinases family and predominantly mediates the signaling for type I/II cytokine receptors, whose ligands include multiple interleukins,

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type I/type II interferon, interleukin-10-related cytokines, and so on.[10] Therefore, the JAK1 signal pathway plays important roles in immune cell-mediated inflammatory reaction, and targeting the JAK1 pathway has become a novel and attractive therapeutic strategy for inflammatory diseases. GLPG0634, an orally available JAK1 inhibitor, displays highly selective inhibition for JAK1, which helps to improve dose tolerance and therapeutic efficacy while avoiding JAK2-related side effects.[11] It has been developed for clinical therapy of Crohn's disease[12] and rheumatoid arthritis[13], displaying good tolerability and efficacy.

Based on these insights, we hypothesized that JAK1 inhibition may have a therapeutic potential for POI by limiting inflammation. In this study, we established a mouse model of POI by IM to evaluate the role of JAK1 in POI and determine whether POI can be attenuated by JAK1 blockade using GLPG0634.

Materials and methods

Experimental animals

Healthy male C57BL/6J mice (clean grade, 4–6 weeks of age, average weight 25 g) were obtained from Hayes Lake Animal Experiments, LLC (Shanghai, China). All mice were housed in separate, pathogen-free cages containing wood shavings, under standardized conditions of temperature and humidity, in a 12-hour light/dark cycle, and provided free access to standard mouse food and tap water.

Ethics statement

Experimental animal protocols were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Fujian Medical University. All animal experiments conformed to the National Animal Welfare Law of China.

Establishment of POI model

Surgical manipulation of the intestine mimicking surgical handling of the intestine during abdominal surgery was performed to establish a mouse model of POI, as previously described.[7] Briefly, after anesthesia, the abdomen was cut open with a median incision, and the small intestine was gently lifted outside the abdominal cavity and placed on a wet gauze pad. Using two moist medical cotton swabs, the whole small intestine was softly inspected along the intestinal wall 3 times for 10 minutes. The gut was manipulated only by applicators without direct touch. After the manipulation, the small intestine was replaced into the peritoneal cavity and the incision was sutured. This operation was performed under aseptic conditions. Normal mice that underwent anesthesia and laparotomy for 10 minutes but without IM served as the sham operation group.

Experimental groups and small-bowel preparation

Eighty-eight mice were randomly allocated to 4 groups ($n = 22$ per group): (1) control group: sham + placebo (physiological saline); (2) IM + placebo; (3) sham + JAK1 inhibitor GLPG0634, 50 mg/kg (Selleck Chemicals, Houston, TX); and (4) IM + GLPG0634. The GLPG0634 and equivolume physiological saline were administrated by oral gavage 30 minutes before the operation. Three hours after the operation, 11 mice from each group were euthanized. The entire jejunum was cut down and was evenly divided into 3 segments (from oral to anal side, named proximal jejunum, mid-jejunum, and distal jejunum, respectively). The mucosa and submucosa of the distal jejunum were removed and discarded under a microscope. Then, the mucosa-free specimens were frozen and stored at −80°C for proinflammatory gene detection and Western blotting. Twenty-four hours after the operation, the remaining mice (11 per group) underwent in vivo survey of intestinal transit, and then were euthanized. Small-bowel specimens were prepared for in vitro studies. The bowel segments harvested from the mid-jejunum were immediately subjected to the examination of muscular contractile activity in vitro. The distal jejunum was frozen and preserved at −80°C for myeloperoxidase (MPO) immunohistochemical staining, and MPO enzyme-linked immunosorbent assay (ELISA).

MPO immunohistochemistry

MPO-positive cells in the small intestine wall were detected by MPO immunohistochemical staining. Briefly, the harvested distal jejunum was immersed in 4% paraformaldehyde solution, embedded in paraffin, and sectioned. After paraffin was removed using xylene, the sections were rehydrated with decreasing concentrations of ethanol. Endogenous peroxidases were ablated with 3% H_2O_2 at room temperature. After washing with tris-buffered saline, the slides were incubated with normal goat serum at 37°C for 20 minutes. Then, diluted, rabbit anti-mouse MPO antibody (Santa Cruz Biotechnology, Dallas, TX) was added and the slides were incubated at 4°C overnight. The sections were incubated with biotinylated, goat anti-rabbit IgG (Santa Cruz Biotechnology, Dallas, TX) for 30 minutes at 37°C. Diaminobenzidine served as the chromogen. The sections were covered with cover glass. Photomicrographs were taken under an OLYMPUS BX43 optical microscope (×400 magnification) (Olympus Corporation, Shinjuku, Tokyo, Japan).

MPO quantification

The MPO within the lamina muscularis of the distal jejunum was quantified 24 hours after surgery using ELISA. Briefly, the frozen, mucosa-free jejunal segments were thawed and homogenized in ice-cold lysis buffer. The homogenates were then centrifuged, and the content of MPO in the supernatants was measured using a mouse MPO ELISA kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China) in accordance with the manufacturer's instructions. The total extracted protein and MPO level per sample were determined.

Proinflammatory gene expression

Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) was performed to detect the mRNA expression of proinflammatory genes such as macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemotactic protein-1 (MCP-1), 3 hours after the operation. The total RNA was extracted from frozen jejunum segments using Trizol (Invitrogen, Carlsbad, CA) and reverse-transcribed with a Qiagen RNeasy Mini Kit (Qiagen Inc, Hilden, Germany) according to the manufacturer's instructions. The complementary DNA was used for qRT-PCR with QuantiFast SYBR Green PCR Kit (Qiagen, Hilden, Germany). Relative expression was shown against mRNA expression of the control sample. The primer sequences used were as follows:

MIP-1α: forward primer, 5'-TTGCTCCCAGCCAGGTGT-3';
reverse primer, 5'-CAGGCATTCAGTTCCAGGTCA-3';
TNF-α: forward primer, 5'-ACTGAACTTCGGGGTGATCG-3';
reverse primer, 5'-CCACTTGGTGGTTTGCTACG-3';
IL-6: forward primer, 5'-CTTGGGACTGATGCTGGTGA-3';
reverse primer, 5'-TTGGGAGTGGTATCCTCTGTGA-3';
ICAM-1: forward primer, 5'-ACTGAACTTCGGGGTGATCG-3';
reverse primer, 5'-CCACTTGGTGGTTTGCTACG-3';

MCP-1: forward primer, 5'-ACCTGGATCGGAACCAAATG-3';
reverse primer, 5'-GTGGTTGTGGAAAAGGTAGTGG-3';
GAPDH: forward primer, 5'-TGGCCTTCCGTGTTCCTAC-3';
reverse primer, 5'-GAGTTGCTGTTGAAGTCGCA-3'.

ELISA was performed to detect the protein expression of proinflammatory genes. MIP-1α, TNF-α, IL-6, ICAM-1, and MCP-1 mouse ELISA kits were used. The methods were the same as those already described for MPO quantification.

Functional studies

Functional studies of the small intestine were undertaken 24 hours after the operation. The mechanical activity of smooth muscle was assessed in vitro using circular muscularis strips of the mid-jejunum, as previously described.[14] Briefly, mid-jejunum segments were immediately immersed in oxygenated Krebs-Ringer buffer at 4°C. Then, the segments were cut into muscularis strips parallel to the circular muscle fiber. The strips were hung in vertical organ chambers and tied to an isometric force transducer (Taimeng Company, Chengdu, China). After acquisition of the spontaneous contractility for 30 minutes, increasing concentrations of the muscarinic agonist bethanechol (0.1, 1, 10, 100, 300 μmol/L) were used to generate concentration-response contractile curves for 10 minutes. The strips were rinsed with Krebs-Ringer buffer for 10 minutes before the different concentrations of bethanechol were replaced. The contractile activity of the muscle strips was recorded and analyzed with the BL-420S biological and functional experimental system (Taimeng Company, Chengdu, China). The contractions were normalized and expressed as g/mm^2/s.

Intestinal transit was evaluated in vivo by surveying the displacement of Evans blue via oral administration, as previously described.[15,16] Briefly, 0.1 mL dye (50 mg Evans blue dissolved in 1 mL physiological saline) was administered via an orogastric cannula. After 20 minutes, the mice were euthanized. The migration distance of the Evans blue dye was surveyed between the pylorus and the achieved point of the dye to evaluate the intestinal transit. The data were expressed in centimeters (cm).

Western blotting

JAK1 activation was analyzed 3 hours after IM and in the sham-operated control group ($n = 6$). The tissue samples were homogenized in lysis buffer, sonicated, and centrifuged. The protein lysate was separated on sodium dodecyl sulfate polyacrylamide gels. After polyacrylamide gel electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Burlington, MA). Then, primary polyclonal antibodies against phospho-JAK1 (Y1022, 1:1000) (Santa Cruz Biotechnology, Dallas, TX), JAK1 (1:1000) (Santa Cruz Biotechnology, Dallas, TX), and secondary antibody (goat anti-rabbit-HRP, 1:2000) (Santa Cruz Biotechnology, Dallas, TX) were used according to the manufacturer's instructions. After rinsing with tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with chemiluminescence reagent BeyoECL Plus) (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China), until the bands could be seen clearly. Chemiluminescence signals were detected with a ChemiDoc XRS+ System (Bio-Rad, Hercules, CA).

Data analysis

The data were represented as mean ± standard deviation. SPSS 16.0 software (IBM, Inc, Armonk, NY) was used for statistical analysis. Student's *t*-test and one-way analysis of variance were applied. Statistical significance was assumed at $P < .05$.

Results

General observations

The mice in all groups recovered from the operation with no deaths or complications (eg, hemorrhage, necrosis, or peritonitis). Moreover, no mechanical intestinal obstruction was observed when the abdomen was reopened and the specimens were harvested post surgically.

JAK1 activation

JAK1 is critical for the signal transduction of many inflammatory cytokines and is closely associated with the inflammatory process. We tested for JAK1 activation post operation in the intestinal wall. As shown in Fig 1, the expression of total JAK1 among the 4 groups showed no statistical difference. Phosphorylated JAK1 protein was barely detected in the control group and the sham + GLPG0634 group, and the difference between the 2 groups was not significant. However, phosphorylation of JAK1 protein was evidently increased after operation in the IM group versus the sham-operated group. In GLPG0634-treated mice, upregulation of JAK1 phosphorylation by IM was decreased compared with the placebo group.

Proinflammatory gene expression

We analyzed the effect of JAK1 inhibitor on the expression of proinflammatory genes such as MIP-1α, IL-6, TNF-α, MCP-1, and ICAM-1 in the muscularis externa 3 hours after operation. As shown in Fig 2, *A*, expression of MIP-1α mRNA increased by 72.4 ± 11.2-fold after IM. Preoperative oral administration of GLPG0634 led to a significant decrease in MIP-1α expression by 25.6 ± 8.1-fold. Therefore, GLPG0634 significantly downregulated MIP-1α mRNA expression post operation. We measured the levels of TNF-α and IL-6 mRNA expression, mostly derived from activated macrophages, and found basal cytokine expression in the control mice. IM led to a 16 ± 2.5-fold upregulation of TNF-α and a 78.2 ± 9.5-fold upregulation of IL-6. In contrast, GLPG0634 pretreatment significantly decreased the cytokine expression after IM by 5.8 ± 1.9-fold for TNF-α and 42.1 ± 10.7-fold for IL-6. We also detected the mRNA expression of adhesion molecule ICAM-1 and chemokine MCP-1. In the IM group, ICAM-1 mRNA was upregulated by 9.3 ± 1.7-fold, but GLPG0634 treatment significantly decreased ICAM-1 expression by 5.4 ± 1.8-fold after IM. MCP-1 mRNA was also upregulated by IM, which was significantly diminished by GLPG0634 treatment (40.3 ± 7.2-fold vs 32.9 ± 5.6-fold). Moreover, we found that the mRNA expression of these 5 genes in the sham + GLPG0634 group was not different from that in the control group. Overall, GLPG0634 markedly decreased the proinflammatory cytokine mRNA expression after IM. To verify these findings, we performed ELISA analysis. A similar pattern of proinflammatory cytokine protein expression was observed (Fig 2, *B*).

Neutrophil infiltration

Surgical trauma of the intestine can cause enormous neutrophil influx into the muscularis. First, we performed MPO immunohistochemistry staining to observe MPO-positive cells in the muscularis externa. As shown in Fig 3, there were very few MPO-positive cells in the control group and the sham + GLPG0634 group. MPO-positive cells that were primarily located in the intestinal muscularis were markedly increased after IM. However, these cells were decreased in the mice in the GLPG0634 pretreatment group (Fig 3, *A*, *B*, *C*, and *D*). To further demonstrate these findings, we conducted a MPO ELISA assay. The data showed that surgical handling caused a

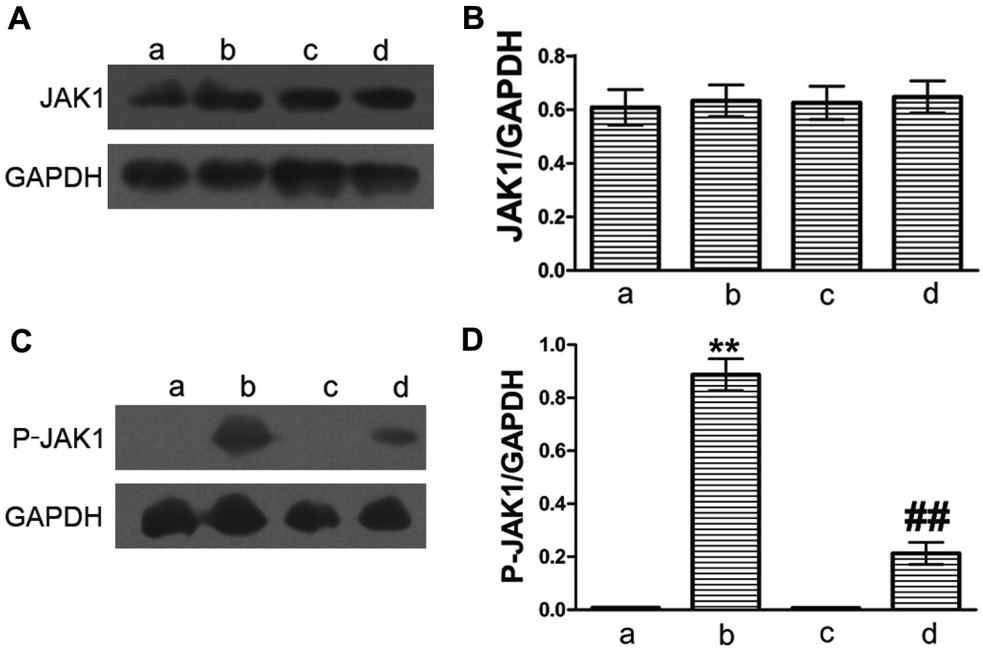

Fig 1. JAK1 activation was analyzed by Western blot in the following 4 groups ($n = 6$ per group): a, control; b, IM + placebo; c, sham + GLPG0634; and d, IM + GLPG0634. *(A)* and *(B)*. The expression of total JAK1 among the 4 groups showed no statistical difference. ANOVA was applied for statistical analysis. *(C)* and *(D)*. Typical Western blot graphics of JAK1 activation in the 4 groups. Phosphorylation of JAK1 was significantly upregulated in the muscularis externa after IM compared with the control group ($^*P < .05$; $^{**}P < .01$). Preoperative GLPG0634 treatment decreased the upregulation of JAK1 phosphorylation compared with the IM + placebo group ($\#P < .05$; $\#\#P < .01$). GAPDH served as an internal control. Student's *t*-test was applied for statistical analysis.

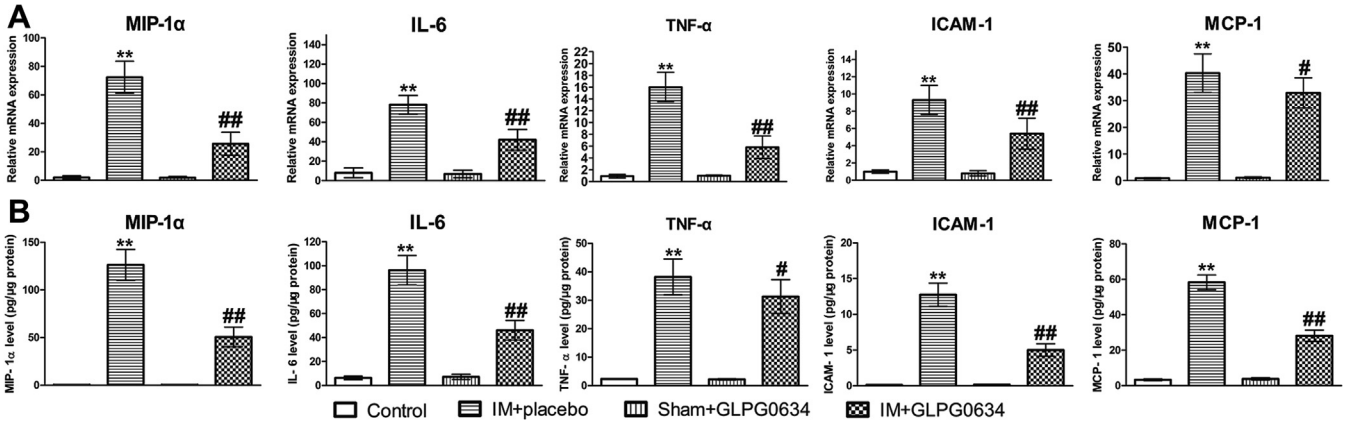

Fig 2. Modulation of proinflammatory cytokine gene expression. *(A)* mRNA expression of MIP-1α, TNF-α, IL-6, ICAM-1, and MCP-1 in muscularis layers was determined by qRT-PCR. *(B)* Protein expression was assessed by ELISA. Four groups were analyzed ($n = 11$ per group): control, IM + placebo, sham + GLPG0634, and IM + GLPG0634. Not only in the mRNA level but also in the protein level, all genes displayed marked upregulation in the IM + placebo group compared with the control groups ($^*P < .05$; $^{**}P < .01$). The administration of JAK1 inhibitor significantly decreased the expression of these genes compared with the IM + placebo group ($\#P < .05$; $\#\#P < .01$). The difference of gene expression between the control and sham + GLPG0634 group was not significant. Student's *t*-test was applied for statistical analysis.

marked upregulation of MPO in the muscularis, with an average level of 67.2 ± 4.1 pg/μg protein. Nevertheless, GLPG0634 pretreatment was effective in decreasing the MPO level, with an average level of 32.5 ± 3.4 pg/μg protein (Fig 3, *E*). The MPO level in the control group and the sham + GLPG0634 group shows no significant difference.

Functional study of intestinal smooth muscle

Intestinal inflammation induced by IM damages smooth muscle function, which subsequently delays intestinal transit in POI. To assess the effect of pharmacological inhibition of JAK1 on intestinal dysmotility induced by IM, we measured mechanical activity of the circular muscularis in vitro and intestinal transit in vivo.

The circular smooth muscle specimens were used to analyze the spontaneous and bethanechol-stimulated muscle contractile activity in vitro. Figure 4, *A* delineates the typical contractile waves of intestinal smooth muscle in response to 100 μmol/L bethanechol. Muscular strips from the controls exhibited tonic contractions accompanied by phasic contractions. GLPG0634 pretreatment did not change the contractile responses of the sham-operated strips. After manipulation, the extent of the tonic contraction was decreased, and the frequency of phasic contractions was also decreased. Nevertheless, contractile responses in strips taken from the mice treated with GLPG0634 were improved. Figure 4, *B* depicts

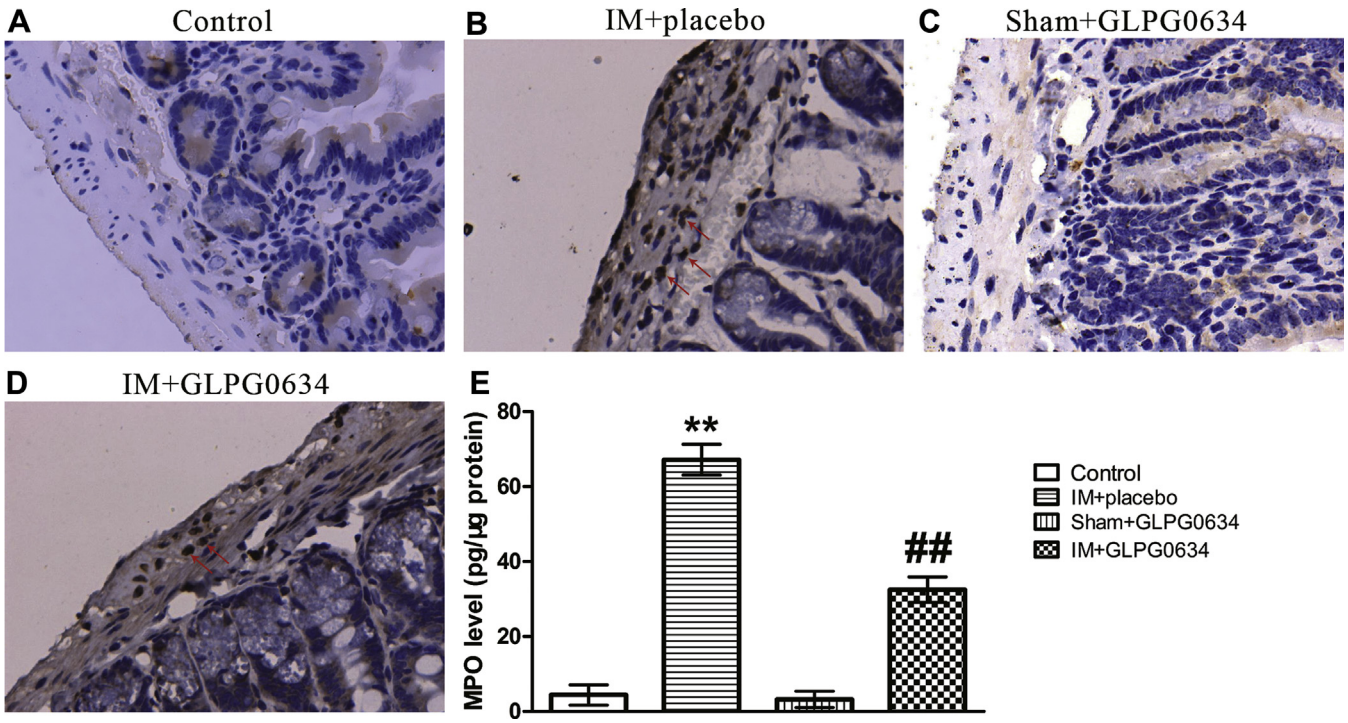

Fig 3. Neutrophil infiltration in muscularis layers. MPO immunohistochemistry and MPO ELISA assay were performed in the following 4 groups ($n = 11$ per group): control, IM + placebo, sham + GLPG0634, and IM + GLPG0634. *(A)* MPO-positive cells were barely observed in the control group. *(B)* MPO-positive cells were markedly increased in the IM + placebo group. *(C)* MPO-positive cells were rarely detected in the sham + GLPG0634 group. *(D)* MPO-positive cells in the IM + GLPG0634 group were much fewer than those in the IM + placebo group. *(E)* The level of MPO in the muscularis externa was significantly upregulated in the IM group compared with the control group ($^{*}P < .05$; $^{**}P < .01$). The level of MPO in the IM + GLPG0634 group was significantly lower than that in the IM + placebo group ($\#P < .05$; $\#\#P < .01$). The MPO level of the sham + GLPG0634 group versus that of control group had no significant difference. Student's *t*-test was applied for statistical analysis.

contractile curves of smooth muscle stimulated with different concentrations of bethanechol in the 3 groups. Obviously, stimulation with bethanechol led to a concentration-dependent enhancement of muscular contractile activity in each group. At the same concentration of bethanechol stimulation, muscle contractility after manipulation was significantly decreased compared with the control groups (at 100 μmol/L bethanechol, 1.88 ± 0.34 g/mm^2/s vs 0.92 ± 0.32 g/mm^2/s). However, the impaired muscular contractile force stimulated by bethanechol was greatly improved by pretreatment with GLPG0634 (1.55 ± 0.29 g/mm^2/s).

Intestinal transit is an important parameter for evaluating intestinal motility. We measured in vivo intestinal transit by analyzing the migration distance of Evans blue in the intestinal tract. As illustrated in Fig 4, *C*, the migration distance was not significantly different between the control group and the sham + GLPG0634 group (16.2 ± 0.81 cm vs 16.8 ± 0.75 cm). IM significantly delayed the transit to 8.3 ± 0.6 cm. Preoperative treatment with GLPG0634 improved the intestinal transit to 13.2 ± 1.5 cm, which was significantly different from the placebo IM group.

Discussion

In the present work, we demonstrated that JAK1 activation mediated resident macrophage activation, subsequent complex inflammatory cascade, and gut dysfunction after IM in mice. JAK1 inhibition alleviated macrophage activation as well as the postoperative inflammation and improved the intestinal dysmotility.

POI remains a major clinical challenge for surgeons, and there is an urgent need for effective methods of preventing or treating POI clinically. Numerous strategies targeted against the local inflammatory process have been devised to prevent POI in the past 2 decades. Some of them suppressed the activation of bowel immune cells such as macrophages[8] or mast cells[17]; whereas others directed proinflammatory cytokines such as IL-1[18], IL-6[19]; adhesion molecules, and chemotactic factors such as ICAM-1,[20] MMP-9,[21,22] and MCP-1[23]; and inhibitory dynamic media such as prostaglandins[5] and nitric oxide (NO).[24] Although all these strategies showed advantageous effects on mitigating POI in animals, they either inhibited a unitary element of the inflammation reaction or interfered with the inflammation reaction at the developing steps. However, the inflammation cascade in POI is complex, and treatment at the onset of intestinal inflammatory reaction might be more valid than treatment after the inflammation cascade is well established.

A strategy that might be more effective for limiting inflammation of POI is targeting upstream signal transduction pathways, which could govern the complex inflammatory cascade. p38 inhibition can significantly inhibit macrophage activation and is shown to be more effective in preventing POI.[9] Moreover, regulation of signaling pathways such as Syk,[25] MK2,[15] and HuR[26] also effectively improved dysfunction of the bowel. Another promising signal pathway for POI prevention is JAK1, which participates in the development of several inflammatory diseases such as rheumatoid arthritis[27] and Crohn's disease.[12] However, the role of this kinase in postoperative intestinal inflammation remains unclear. In this study, we found that intestinal injury resulted in a marked activation of JAK1. GLPG0634 is a novel compound whose selectivity for JAK1 is 30 times higher than that for JAK2.[11] Here, we observed GLPG0634 pretreatment significantly impeded muscular JAK1 activation following intestinal operation.

Activation of resident macrophages within the intestinal muscularis is a key factor for the onset of postoperative inflammation.[8] A recent study demonstrated that mechanical insult promotes Th1 cell differentiation into Th1 memory cells secreting IFN-γ, which

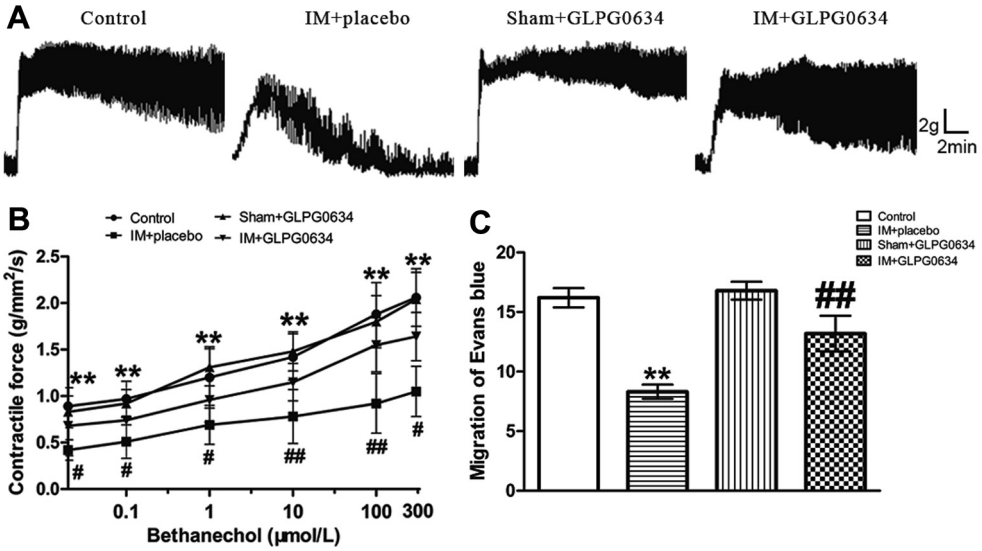

Fig 4. Functional study of smooth muscle in vitro and in vivo ($n = 11$ per group). *(A)* Typical waves of intestinal circular smooth muscle contractile responses to 100 μmol/L bethanechol. *(B)* Curves of smooth muscle contractile activity stimulated with increasing concentrations of bethanechol in the following groups: control, IM + placebo, sham + GLPG0634, and IM + GLPG0634. *(C)* Study of in vivo intestinal transit. Intestinal transit was significantly damaged in the IM mice compared with the control mice. Intestinal transit was significantly repaired by JAK1 inhibitor treatment compared with the IM + placebo group. The sham + GLPG0634 group displayed no statistical difference from controls. $^{*}P < .05$; $^{**}P < .01$, compares IM + placebo group with the control mice; $\#P < .05$; $\#\#P < .01$, compares IM + GLPG0634 group with the IM + placebo mice. Student's *t*-test was applied for statistical analysis.

ultimately activates the intestinal resident macrophages, triggering local inflammation.[28] Of note, the involved IFN-γ receptor signal is absolutely dependent on JAK1.[10,29] Therefore, we speculated that JAK1 potentially served as a core regulator for resident macrophage activation in POI. To verify this hypothesis, we detected macrophage activation marker MIP-1α gene expression. Our data showed that JAK1 predominantly regulates resident macrophage activation early after IM, indicating that inhibition of JAK1 can restrict the postsurgical inflammation at the initial step.

Resident macrophage activation is accompanied by inflammatory cascade, in which proinflammatory cytokines play crucial roles. Among these cytokines, a prototypical one is IL-6. As is known, IL-6 exerts its functions in regulating target gene transcription mainly employing gp130/JAK1/STATs signaling.[30] These target genes include a wide range of proinflammatory cytokines. In POI models, IL-6 promoted upregulation of the adhesion molecule ICAM-1 as well as the chemokine MCP-1, augmenting leucocyte recruitment.[19,31] Of importance, IL-6 expression was at the maximum level at 1 hour after IM and sustained elevated levels up to 12 hours,[19] which indicates that IL-6 can regulate proinflammatory cytokine expression not only in the early phase but also in the late phase of the postoperative inflammatory process. IL-6 is a key cytokine promoting the inflammation cascade that is well established to result in POI. As an important medium molecule of IL-6 receptor signal, JAK1 acts as a dominant modulator in the postoperative inflammatory cascade, which can be demonstrated by our data that inhibition of JAK1 notably decreased the IM-induced upregulation of proinflammatory cytokine expression and neutrophil infiltration.

The inflammatory condition induced by IM leads to impaired intestinal motility and, ultimately, POI. The extent of inflammation is positively correlated with the degree of intestinal motor disorder.[32] It has been demonstrated that inhibitory dynamic media such as NO and prostaglandins are mainly liberated by activated, muscularis-resident macrophages, and infiltrated leukocytes directly suppress the postsurgical function of smooth muscle by acting on gut neurons.[24] Here, we found that GLPG0634 was effective in alleviating postoperative gut motor dysfunction, displaying enhanced contractile function of smooth muscle and improved intestinal transit. Of note, we also found that although GLPG0634 could not totally repair the IM-induced impairment of muscular contractility, it improved the profile of contractile waves to be similar to those of the control muscle. Despite the lack of data of inhibitory dynamic media production in our work, it seems possible that the improved intestinal function after IM is due to the decreased inhibitory dynamic media, as their cellular source decreased by JAK1 inhibition. This presumption certainly needs further study to be clarified.

Overall, our study showed for the first time that JAK1 functions as a core regulator in the reticular inflammatory cascade resulting in POI, and JAK1 inhibition shows a positive effect on treating POI. However, interference with the immune response may result in side effects. Since JAK1 is known to be important in immune regulation, further studies are required to evaluate whether JAK1 inhibition would lead to adverse events such as anastomosis fistula, delayed wound healing, or infection in POI models. Moreover, colonic morbidity is also an important parameter for clinical postoperative ileus. Therefore, the effect of JAK1 inhibition on postoperative colonic morbidity should be investigated in the future.

In conclusion, this study demonstrated that an intervention with JAK1 inhibitor improved POI by alleviating macrophage activation as well as the postoperative inflammation. These findings suggested that JAK1 inhibition could be a prospective strategy for prevention or mitigation of POI, and preoperative oral administration of GLPG0634 might be a convenient approach for application.

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Conflict of interest/Disclosure

The authors declare that there are no conflicts of interest.

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