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Article

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Posted Date: October 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10293537/v1

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Additional Declarations: No competing interests reported.

The interaction between PAK1, CXCL1, and mechanotransduction during the development of ileus

Short title: PAK1, CXCL1, and mechanotransduction in ileus

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Abstract

Background

Ileus is a well-recognized clinical problem, significantly impacting patient outcomes and increasing patient care costs. The objective of this study was to understand the interactions between PAK1, CXCL1, and mechanotransduction that contribute to the development of ileus.

Methods

Contractile activity was investigated after ileus was induced by gut manipulation in wild-type and PAK1 knockout mice. Cellular changes were investigated using a cyclical stretching human intestinal smooth muscle cell model (hISMC). Increased stress (as in edematous tissue) was simulated in human intestinal smooth muscle cells (hISMCs) by increased cyclic stretch (ECS). The non-edematous condition was simulated using control cyclical stretch (CCS).

Results

Decreased spontaneous contractile activity was detected after gut manipulation in wild-type but not PAK1 knockout mice. Although intestinal wall inflammation developed after gut manipulation in the wild-type and PAK1 knockout mice, as indicated by increased mast cell protease activity and intestinal edema, several cytokines were selectively reduced in the PAK1 knockout mice compared with wild-type mice, including CXCL1. In hISMCs, CXCL1 decreased MLC and MYPT1 phosphorylation, indicating that CXCL1 reduces intestinal contractile activity via effects on intestinal smooth muscle. Subjecting hISMCs to ECS increased CXCL1 secretion compared with CCS, indicating that CXCL1 is induced via mechanotransductive signals, and the increased CXCL1 secretion was blocked by PAK1 inhibition. NF-κB nuclear translocation increased in hISMCs subjected to ECS compared with CCS, and PAK1 inhibition prevented the increased NF-κB nuclear translocation, indicating that stretch-induced increases in NF-kB activation are PAK1-dependent. Inhibiting NF-κB activity prevented stretch-induced increases in CXCL1 secretion.

Conclusions

These results demonstrate that interactions between mechanotransduction, CXCL1, and PAK1 contribute to ileus. Mechanical signals induce increased PAK1 activity, which activates NF-kB, and increased NF-kB activity induces increased CXCL1 secretion. CXCL1 inhibits intestinal contractile activity. Understanding the mechanism for ileus development will facilitate the identification of new drug targets to treat ileus.

Keywords

Ileus, CXC chemokine 1, p21-activated kinase 1, NF-kappa B, mechanotransduction

Background

Ileus is a significant clinical problem and economic burden affecting a large number of patients, including critically ill[1], trauma,[2] and postsurgical patients[3]. Ileus, defined as depressed or ceased intestinal contractile activity, is a well-recognized clinical problem that significantly impacts patient outcomes by preventing enteral feeding. Many studies, including multiple meta-analyses, demonstrated that early enteral feeding improves outcomes, including mortality, sepsis, overall complications, infectious complications, and multi-organ failure, compared with delayed or parenteral feeding in critically ill patients.[4-7] Furthermore, feeding intolerance due to ileus prolongs hospital and intensive care unit stays and increases infectious and thromboembolic complications.[2, 8] Delayed feeding due to ileus also causes nutritional deficits in already compromised patients, which often necessitates parenteral feeding. Furthermore, ileus causes significant patient discomfort.[9, 10]

In addition to the impact on patient outcomes, the deleterious effects of ileus increase patient care costs. In an Australian study, patient care costs increased by 26% in patients with ileus after colorectal surgery,[11] and in a recent meta-analysis, ileus increased total hospital costs by 66% in patients undergoing abdominal surgeries.[12] Ileus increased individual hospital costs by $7000–$12,000 in the US and by approximately €4000 in Dutch and Danish hospitals.[8, 13, 14] Furthermore, patients with ileus experience a significant reduction in quality of life, even 3 months after surgery.[8] Although many surgical, trauma, and critically ill patients experience ileus, the available drugs to treat ileus are not very effective.[15] In a retrospective study of moderate-to-severe trauma patients, treatment with prokinetics did not reduce gastric residual volumes or time to feeding goal.[2] Therefore, new drugs to treat ileus are needed, and understanding the mechanisms contributing to the development of ileus is important for identifying new drug targets for the treatment of ileus.

We recently showed that P21-activated kinase (PAK1) activity is increased in intestinal smooth muscle tissue in an ileus model, and increased PAK1 activity inhibits intestinal motility.[16] In a primary intestinal smooth muscle cell model, increased cyclical stretch increased PAK1 activity, resulting in decreased myosin light chain (MLC) phosphorylation via decreased myosin phosphatase target subunit

(MYPT1) phosphorylation.[16] Inhibition of PAK1 in intestinal smooth muscle cells subjected to increased cyclical stretch restored MYPT1 phosphorylation levels.[16] These data indicate that mechanotransduction mediates at least part of the decreased intestinal motility induced by intestinal edema and the development of ileus. We also showed that CXCL1 levels increase in intestinal smooth muscle tissue after the induction of ileus, and circulating CXCL1 levels are significantly higher in trauma patients who develop ileus compared with trauma patients who do not develop ileus.[17] Furthermore, CXCL1 inhibits intestinal contractile activity.[17] Thus, PAK1, mechanotransduction, and CXCL1 all contribute to ileus; however, the interactions between these signaling elements during the development of ileus are unclear. The objective of the present study was to understand the interactions between PAK1, CXCL1, and mechanotransduction during the development of ileus using a PAK1 knockout model and cyclical stretching of primary hISMC.

Methods

Animal experiments

All animal experiments complied with the Institutional Care and Use Committee Guidelines, and the protocol was approved at both institutions (HSC-AWC-13-090 and DEMAB/44-1/2025). All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The PAK1 knockout mice (C57BL/6) were derived at the Medical Gene Technology Unit at the Institute of Experimental Medicine in Budapest, Hungary. PAK1 knockout was verified via western blotting. Heterozygotes were bred with heterozygotes to obtain PAK1 knockout mice, and the wild-type littermates were used for comparisons. Male and female mice (3–6 months; C57BL/6) were used for all experiments. Mouse weights were measured weekly for 12 weeks. At the time of sacrifice, intestinal lengths were measured.

Mice in each genotype group were randomized into the GM or SHAM groups. Ileus was induced using a gut manipulation (GM) model.[18] Briefly, the mice were anesthetized with isoflurane. The small intestines were exteriorized via a laparotomy and rolled 3 times along the entire length of the small intestine between two sterile cotton swabs soaked in saline. The intestines were returned to the abdominal cavity, and the abdomen was closed. The SHAM group was subjected to a laparotomy only. At the end of the study period, mice were euthanized by cervical dislocation.

Contractility measurements

Contractile activity was measured in an organ bath system with external force displacement transducers (Experimetria Ltd., Budapest, Hungary) connected to a PowerLab (AD Instruments, Colorado Springs, CO, USA). Data were recorded and analyzed using LabChart software (AD Instruments, Colorado Springs, CO, USA). Full-thickness intestinal strips (1 cm) from the terminal ileum, excluding the last 1 cm close to the cecal junction, were mounted in the organ bath chambers containing oxygenated (95:5 O_2:CO_2) Krebs buffer, and the tension was adjusted to 0.5 g. After equilibrating for 10 minutes, the tension was readjusted to 0.5 g and equilibrated for another 20 minutes. Baseline contractile activity was recorded for 10 minutes, followed by increasing concentrations of carbachol (10^{-8} to 10^{-4} M) added directly to the organ bath at 5-minute intervals. Agonist-induced contractile activity was defined as the contractile activity (integral) in response to carbachol. The following contractile activity measurements were calculated using the AD Instruments software: contractile activity as the integral (from zero), contractile activity as the integral from the minimum, contractile amplitude (difference between the minimum and maximum for each cycle), contractile tone calculated as the average minimum of the contraction cycle, and maximum agonist-induced (carbachol) contractile activity. All measurements were averaged over 4 minutes of data and normalized to tissue weight. Carbachol-induced contractions were normalized to baseline.

Western blotting and ELISAs

Western blotting was performed as previously described.[16] The PAK1, MLC, and phosphor-MLC (p20) antibodies were purchased from Cell Signaling. The GAPDH antibody was purchased from

Millipore. The MYPT1 and phospho-MYPT1 (Thr 696) antibodies were purchased from BD Biosciences and Millipore (Billerica, MA), respectively. The M2 muscarinic receptor antibody was purchased from Invitrogen (ThermoFisher), and the M3 muscarinic receptor antibody was purchased from Almone Laboratories. Western blots were analyzed using ImageJ. MLC and phospho-MLC were analyzed on separate western blots and normalized to the respective GAPDH levels for each blot. Therefore, MLC phosphorylation was calculated as follows: (MLC-P/GAPDH)/(MLC/GAPDH).

MCP-1, CXCL1, and mast cell protease were measured using ELISA kits from R&D Systems, Invitrogen, and RayBiotech, respectively, following the manufacturers' instructions.

NF-κB Activity

Human ISMCs subjected to stretch protocols (CCS, ECS) were sonicated and centrifuged to obtain pellets and supernatants for nuclear protein extraction and quantification. Nuclear proteins were isolated using an Abcam nuclear extraction kit following the manufacturer's protocol. Nuclear factor (NF)-κB activity was assessed in nuclear extracts using a RayBiotech NF-kB assay kit and normalized to protein concentration determined by BCA (Invitrogen). Absorbance was measured at 450 nm using a Bio-Rad spectrophotometer.

Cytokine arrays

Cytokine and chemokine levels in the mouse ileal smooth muscle were measured using a mouse cytokine array (R&D Systems, Minneapolis, MN) following the manufacturer's directions. The cytokine array kit included capture antibodies for 40 cytokines and chemokines immobilized on nitrocellulose membranes. Antibody binding was quantified using ImageJ.[19, 20]

Wet-to-dry weight ratios

Wet-to-dry weight ratios were measured in the mid-section of the small intestine. Intestinal samples were weighed immediately after collection. After drying in a 65°C oven, samples were weighed again. Wet-to-dry weight ratios were calculated as [(wet weight) – (dry weight)]/dry weight.

Cell experiments and stretching protocols

Disease-free small intestinal tissue was collected from organ donor patients after obtaining informed consent from a family member and approval by the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston. All tissue collection was performed in accordance with the Declaration of Helsinki. Human ISMCs were isolated and characterized, as previously described.[16] The hISMCs were cultured in smooth muscle cell growth media (Cell Application Inc., cat 311-500, USA) and used at passages 4–5.

The hISMCs were cultured in 6-well BioFlex® culture plates with flexible silicone elastomer bottoms (Flexcell, Hillsborough, NC) after coating the plates with 1 mg/ml poly-d-lysine (Sigma). Equibiaxial stretching was performed using the FX-4000^{TM} Tension System (Flexcell, Hillsborough, NC). The cyclical stretch protocols were designed to mimic the amplitude and frequency measured *in vivo* in previous experiments.[16] Control cells were subjected to basal cyclical stretch consisting of 0.4-Hz sinusoidal strain with a minimum 1% and a maximum 3% elongation. This stretching protocol, which mimics basal spontaneous intestinal contractions, is referred to as control cyclical stretch (CCS). Edema causes tissue swelling, and the edema cyclical stretch (ECS) protocol, which mimics the edema development in the gut manipulation model, consisted of gradually escalating 0.4-Hz cyclical stretch starting at a minimum 1% and a maximum 3% elongation and reaching a minimum 18% and maximum 20% elongation at 30 min, which was maintained for 3.5 h.

THP-1 cells, an immortalized human monocyte-like cell line (ATCC), were seeded onto Flexcell plates, and 30 hours later, the cells were treated with phorbol-12-myristate-13-acetate (PMA, 100 ng/mL). Of note, THP-1 cells grow in suspension but attach to the Flexcell plates and differentiate into macrophage-like cells after PMA treatment. Forty-eight hours after PMA treatment, cells were subjected to the CCS or ECS stretching protocols, as described above.

Human ISMC and THP-1 cells were treated with 10 μM IPA-3 or IMD 0354 for 30 minutes before subjecting the cells to CCS or ECS.

Statistical analyses

All data are reported as means with standard errors. Group sizes were determined using a Power analysis and based on previous experience. Groups were compared using a main effects ANOVA for multiple groups or t-tests for two groups. All statistics were performed using Statistica (v15). $P < 0.05$ was considered significant.

Results

Characterization of PAK1 knockouts

PAK1 knockout in the small intestines was verified by western blotting. PAK1 expression was significantly reduced in heterozygote mice and undetectable in homozygote PAK1 knockout mice compared with wild-type mice (Figure 1A–B). As previously demonstrated in an intestinal edema rat model of ileus,[16] intestinal smooth muscle PAK1 levels increased in the gut manipulation model of ileus compared with the sham-operated controls in wild-type mice (24 hours; Figure 1B). However, gut manipulation did not significantly increase PAK1 levels in the heterozygous or homozygous PAK1 knockout mice (Figure 1B).

Female mice were significantly smaller than male mice for all genotypes ($p < 0.005$). Male PAK1 knockout mice were slightly but significantly smaller than male wild-type mice (ANOVA, $p < 0.05$, +/+ vs. −/−), and female PAK1 knockout and heterozygous mice were 8–10% smaller than female wild-type mice ($p < 0.05$ for both) (Figure 1C). Of note, the PAK1 knockout mice tended to have fewer pups; thus, there are fewer mice in the PAK1 knockout mouse groups. The small intestinal lengths were not significantly different between the wild-type, heterozygotes, and PAK1 knockout mice in the SHAM group (Figure 1D). However, intestinal lengths were shorter in the gut manipulation group (GM group) compared with the SHAM group in the PAK1 knockout mice (Figure 1D). Visually, there were no gross abnormalities in the small intestines of PAK1 knockout mice (data not shown).

Gut manipulation to induce ileus causes mast cell degranulation and the development of edema in the small intestine. Thus, these parameters were used as indicators of effective gut manipulation. As shown in Figure 1E, mast cell protease levels increased significantly in response to gut manipulation in both the wild-type and PAK1 knockout mice 4 hours after gut manipulation to induce ileus (+/+, $p < 0.005$; −/−, $p < 0.05$); however, no significant differences in mast cell protease activity were detected between the wild-type and PAK1 knockout mice after gut manipulation (+/+ GM vs. −/− GM, $p = 0.030$). Wet-to-dry weight ratios, indicating the development of edema, increased significantly 24 hours after gut manipulation in all three genotype groups (Figure 1F). The wet to dry weight ratios were significantly higher in the heterozygous mice compared with the wild-type mice after gut

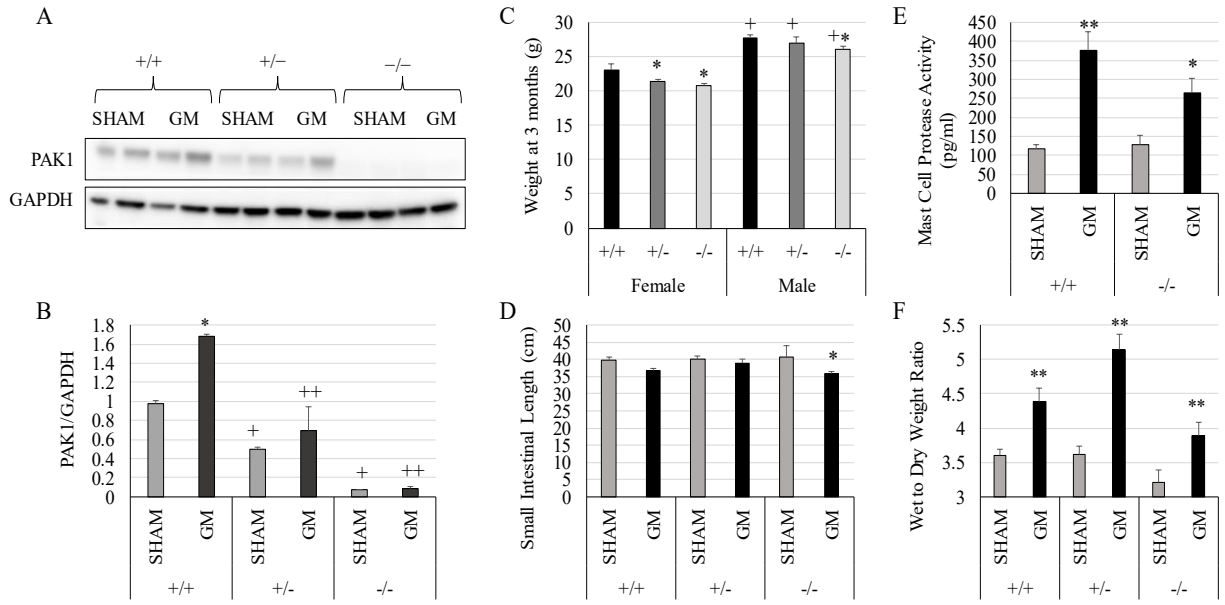


Figure 1. Characterization of PAK1 knockout mice after sham surgery (SHAM) or gut manipulation (GM). A. Western blot showing the expression of PAK1 in wildtype (+/+), heterozygous (+/−), and homozygous (−/−) PAK1 knockout mice. B. Quantification of PAK1 expression 24 hours after SHAM or GM. (n = 2 per group; +, $p < 0.05$ and ++ $p < 0.005$ vs. respective wildtype; *, $p < 0.05$ vs. SHAM in same genotype) C. Body weights in male and female mice from the three mouse genotypes at 3 months of age. (n = 6–24 per group; *, $p < 0.05$ vs. wildtype of same sex; +, $p < 0.05$ vs. female). D. Small intestinal lengths 24 hours after SHAM or GM. (n = 4 per group; *, $p < 0.05$ vs. SHAM in same genotype group). E. Mast cell protease levels in the peritoneal fluid 4 hours after SHAM or GM. (n = 6 per group; *, $p < 0.05$ and ** $p < 0.005$ vs. SHAM in same genotype group). F. Wet-to-dry weight ratios, indicating edema development, 24 hours after SHAM or GM. (** $p < 0.005$ vs. SHAM in same genotype group).

manipulation ($p = 0.043$) and tended to be lower in the PAK1 knockout mice compared with the wild-type and heterozygous mice after gut manipulation (+/+ GM vs. −/− GM, $p = 0.059$; +/− GM vs. −/− GM, $p = 0.060$), suggesting less inflammation in the intestines of the PAK1 knockout mice after gut manipulation compared with the wild-type and heterozygous mice.

Intestinal contractility after the induction of ileus

Contractile activity, including the integral, the integral from the minimum, the amplitude, the tone, and the maximum agonist-induced contractile activity, was measured 24 hours after gut manipulation or sham surgery. Contractile activity, calculated as the integral, was not significantly different between the wild-type, heterozygous, and homozygous mice or between the gut manipulation and sham-operated mice (Figure 2A). However, if contractile activity was calculated as the area under the curve integrated from the minimum signal, contractile activity decreased significantly in wild-type mice (Figure 2B, SHAM +/+ vs. GM +/+, $p < 0.01$), but not in PAK1 knockout mice (SHAM -/- vs. GM -/-, $p > 0.05$), after the induction of ileus (GM group). Contractile activity calculated as the integral from zero is affected by the tone (average minimum of the contractile cycle), and the contractile activity calculated as the integral from the minimum of the contractile cycle is affected by the contraction amplitude. In agreement with the integral data, no significant differences in tone were detected between the mouse genotypes or after gut manipulation (Figure 2C). In contrast, contraction amplitude was significantly decreased after gut manipulation in the wild-type but not the PAK1 knockout mice (Figure 2D), in agreement with the integral from the minimum data. Maximum contractile activity induced by carbachol decreased significantly in all genotypes after gut manipulation, but was not significantly different between the genotype groups (Figure 2E). Frequency was not affected by gut

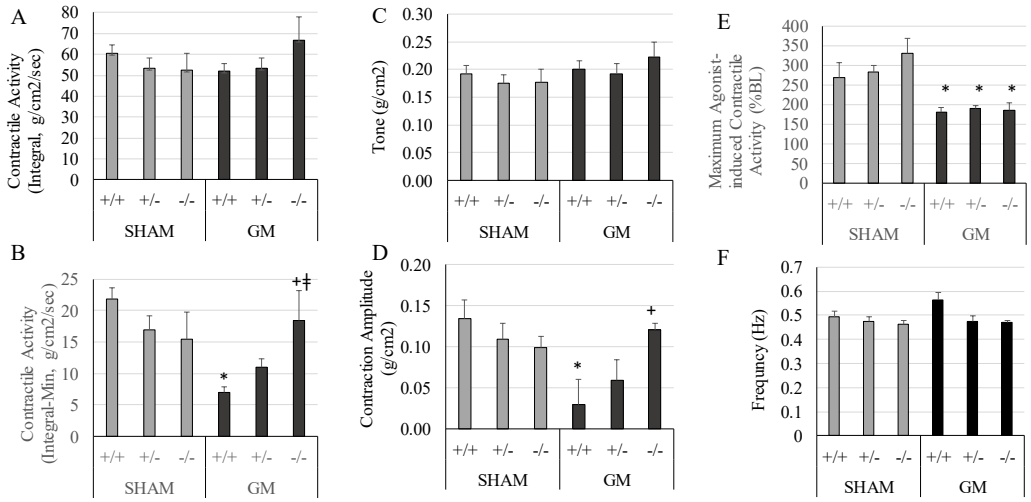


Figure 2. Contractile activity 24 hours after gut manipulation or sham surgery in wildtype, heterozygous, and homozygous PAK1 knockout mice. All contractile activity values reflect the averages over 4 minutes of data. A. Contractile activity, calculated as the integral (from zero). B. Contractile activity calculated as the integral from the minimum value. C. Intestinal tone, calculated as the average minimum value. D. Contraction amplitude, calculated as the average contraction cycle amplitude. E. Maximum agonist-induced contractile activity calculated as the maximum area under the curve after step-wise increased carbachol treatment from 10^{-9}–10^{-4}. (n = 11–12 in wildtype groups, 12–13 in heterozygote groups, and 5 in homozygote groups; *, $p < 0.05$ vs. respective SHAM in same genotype group; +, $p < 0.05$ vs. wildtype in same treatment group; ǂ, $p < 0.05$ vs. heterozygotes in same treatment group). F. Contraction frequency.

manipulation or genotype (Figure 2F). Thus, both spontaneous and agonist-induced contractile activity were decreased after gut manipulation, but PAK1 knockdown only affected spontaneous contractile activity.

Cytokine levels after induction of ileus

Inflammation is thought to be involved in the development of ileus, and Figures 1E and F indicate that gut manipulation induced inflammation. Thus, a panel of cytokines and chemokines was measured in the intestinal smooth muscle tissue collected from wild-type and PAK1 knockout mice 4 hours after subjecting the mice to gut manipulation or sham surgery. As shown in Figure 3 A and B, more cytokines were up-regulated (> 1.5 fold GM/SHAM), and fewer cytokines were down-regulated (< 0.5 fold GM/SHAM) in the wild-type mice compared with the PAK1 knockout mice (+/+ vs. −/−: > 1.5 threshold, 11 vs. 3; < 0,5 threshold, 2 vs. 14; $p < 0.001$ using Chi-square test). TIMP1 and JE/MCP-1 (MCP-1 is the human homolog of the mouse JE) were increased in both the wild-type and PAK1 knockout mice. However, IL-27, CINC-1/CXCL-1 (CXCL1 is the human homolog of mouse CINC-1), SDF-1, TREM1, IL-1β, and RANTES were increased in the wild-type but not the PAK1 knockout mice. The levels of JE/MCP-1, CINC-1/CXCL1, and IL-1β in mouse intestinal smooth muscle tissue

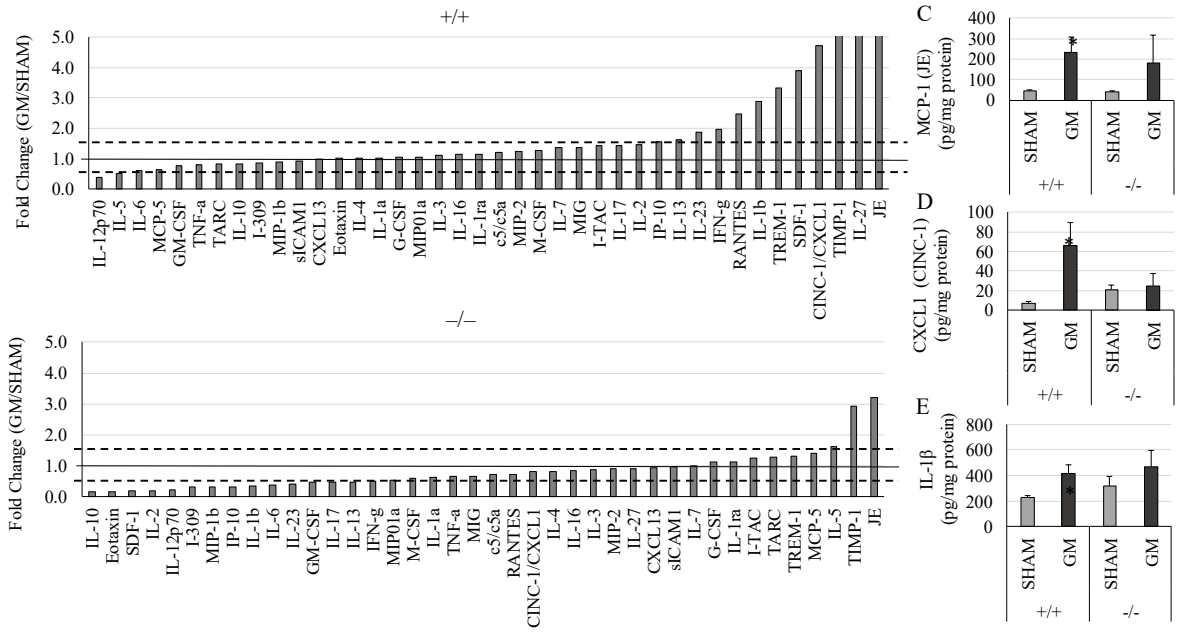


Figure 3. Changes in inflammatory mediators in intestinal smooth muscle tissue 4 hours after gut manipulation or sham surgery in wildtype, heterozygous, and homozygous PAK1 knockout mice. A, B. The ratios of cytokines after sham surgery (laparotomy only, SHAM) versus gut manipulation (GM) were measured using cytokine arrays in wildtype and PAK1 knockout mice, respectively (n = 3 per group). C–D. Changes in MCP-1, CXCL1, and IL1b levels, respectively, after sham surgery (laparotomy only, SHAM) or gut manipulation (GM) in intestinal smooth muscle tissue from wildtype and PAK1 knockout mice. Cytokines were measured using ELISAs. (n = 3–11 per group; *, $p < 0.05$ vs. SHAM).

were verified using ELISAs. Similar to the cytokine array results, MCP-1 increased after gut manipulation in the wild-type mice ($p < 0.001$) and tended to increase in the PAK1 knockout mice ($p = 0.13$) (Figure 3C). In agreement with the cytokine array data, CXCL1 significantly increased in the wild-type but not the PAK1 knockout mice ($p = 0.69$) (Figure 3D). IL-1β significantly increased in the wild-type mice ($p < 0.001$) but not the PAK1 knockout mice ($p = 0.30$) (Figure 3E).

Effects of CXCL1 on contractile activity

To determine if CXCL1 and increased stretch had direct effects on smooth muscle cells, the effects of CXCL1 on MLC phosphorylation, which is an essential step for smooth muscle contraction, were determined. Primary hISMC were subjected to CCS or ECS to mimic control conditions and edema development after gut manipulation. As shown in Figure 4A and B, treatment of hISMC with increasing concentrations of CXCL1 resulted in dose-dependent decreases in MLC phosphorylation in cells subjected to ECS but not CCS. CXCL1 (100 ng/ml) also induced a small but significant decrease in MYPT1 phosphorylation at Thr696 in cells subjected to ECS but not CCS (Fig. 4C, D). MYPT1 is the targeting subunit of myosin phosphatase, and reducing the inhibitory phosphorylation of MYPT1 increases phosphatase activity and, consequently, lowers MLC phosphorylation.

The levels of muscarinic receptors affect intestinal contractile activity; the M2 and M3 muscarinic receptor subtypes are expressed in intestinal smooth muscle cells. Therefore, we determined the effects of CXCL1 and stretch on the expression of M2 and M3 muscarinic receptor levels in hISMC. Increased cyclical stretch (ECS) induced decreased expression of both M2 and M3 muscarinic receptors (Figure 4E–G). However, CXCL1 treatment did not significantly alter M2 and M3 receptor levels.

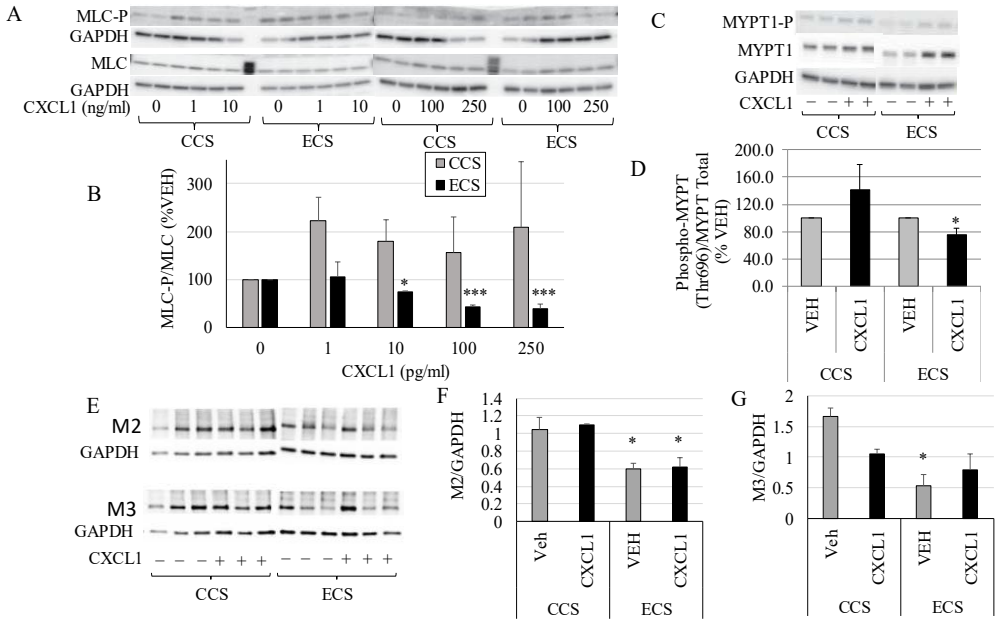


Figure 4 . Changes in myosin light chain (MLC) and MYPT1 phosphorylation and muscarinic receptor levels in primary human intestinal smooth muscle cells after treatment with CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours. A–B. Representative western blots and quantification of changes in the ratio of phospho-MLC to total MLC in response to treatment with varying concentrations of CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours. (n = 2–7 per group; *, $p < 0.05$ vs. CCS; ***, $p < 0.0001$ vs. CCS). C–D. Representative western blots and quantification of changes in phospho-MYPT1(Thr696) to total MYPT1 in primary human intestinal smooth muscle cells in response to treatment with 100 ng/ml CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours (n = 5 per group; *, $p < 0.05$ vs. CCS). E–G. Representative western blots and quantification of changes in M2 and M3 muscarinic receptors in primary human intestinal smooth muscle cells in response to treatment with 200 ng/ml CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours (n = 3 per group; *, $p < 0.05$ vs. CCS).

Role of mechanotransduction in CXCL1 secretion from intestinal smooth muscle cells

Intestinal edema increased significantly in the gut manipulation animal model of ileus (Figure 1F). We previously showed that intestinal edema increases mechanical stretch in the intestinal smooth muscle layers.[21, 22] To determine if increased stretch in the intestinal smooth muscle layers induced CXCL1 secretion, we measured CXCL1 secretion from hISMC after subjecting the cells to CCS, to mimic normal physiological conditions in the intestinal wall, or ECS, to mimic edema development and increased stretch in the intestinal wall. As shown in Figure 5A, subjecting hISMC to increased cyclical stretch (ECS) increased CXCL1 secretion into the media compared with CXCL1 levels under physiological cyclical stretch (CCS).

Although edema (and therefore increased stretching of ISMC) develops in both the wild-type and PAK1 knockout mice, CXCL1 was not increased in the intestinal smooth muscle of PAK1 knockout mice after the induction of ileus (Figure 3D), suggesting that PAK1 influenced CXCL1 secretion. Therefore, we determined the effects of increased stretch on CXCL1 secretion in hISMC in the presence of the PAK1 inhibitor, IPA-3 (10 μM). IPA-3 blocked the stretch–induced (ECS) increase in

CXCL1 secretion but did not affect CXCL1 secretion in hISMC subjected to CCS (Figure 5B and C). Therefore, stretch-induced increases in CXCL1 secretion may be at least partially mediated by PAK1.

NF-κB is induced by cyclical stretch in a number of cell models[23, 24] and is an important transcription factor regulating CXCL expression.[25] Therefore, we determined the effects of increased stretch on NF-κB activity. NF-κB activity was increased in hISMC subjected to ECS compared with CCS (Figure 5D). To determine the effects of PAK1 on NF-κB activity, we measured NF-κB activity in hISMC subjected to CCS or ECS and in the presence or absence of IPA-3. The increased NF-κB activity induced by increased cyclical stretch (ECS) was partially inhibited by 10 μM IPA-3 (Figure 5D). Furthermore, inhibition of NF-κB with IMD 0354 (10 μM) blocked the stretch-induced increase in CXCL1 secretion (Figure 5E), indicating that stretch-induced increases in CXCL1 secretion are mediated by NF-κB. Overall, these results indicate that CXCL1 secretion is induced by increased stretch via NF-κB activation, and PAK1 activity is necessary for the induction of NF-κB.

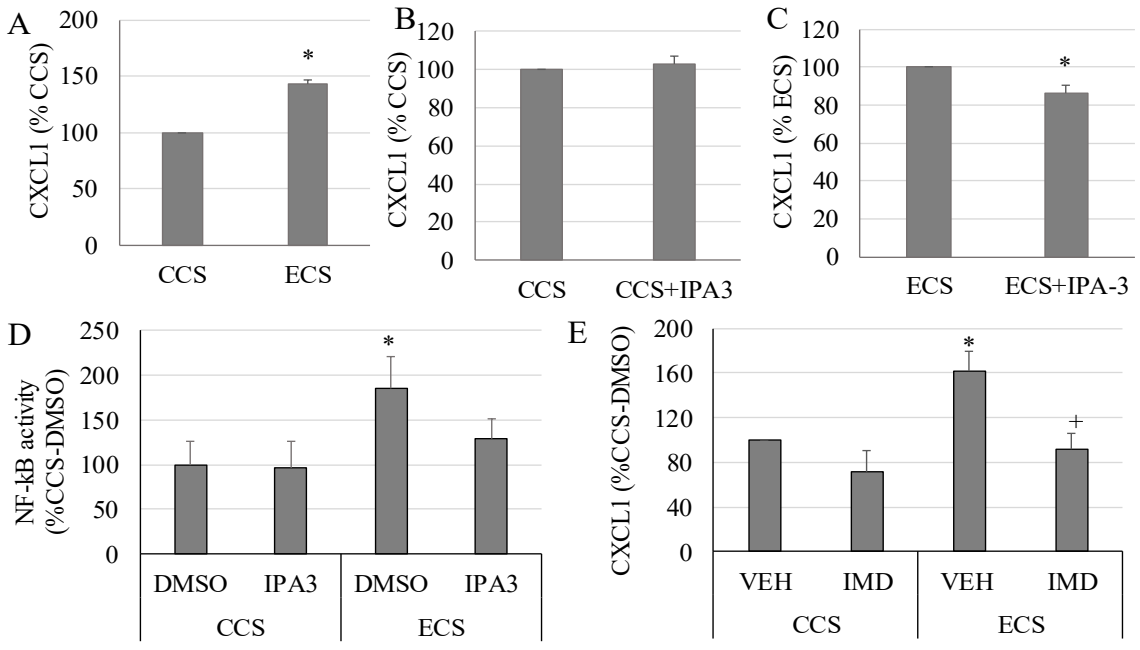


Figure 5. The regulation of CXCL1 secretion from human intestinal smooth muscle cells (hISMC). A. CXCL1 secretion after subjecting hISMCs to CCS or ECS stretching protocols. (n = 6 per group; * $p < 0.05$ vs. CCS). B–C. CXCL1 secretion in the presence or absence of the PAK1 inhibitor IPA-3 (10 μM) and subjecting hISMCs to CCS or ECS. (n = 8 per group; ** $p < 0.05$ vs. VEH). D. NF-kB activity in the presence or absence of IPA-3 and subjecting hISMCs to CCS or ECS. (n = 5 per group; ** $p < 0.05$ vs. CCS/DMSO). E. CXCL1 secretion in the presence or absence of the NF-kB inhibitor IMD (1.5 μM) and subjecting cells to CCS or ECS. (n = 3 per group; * $p < 0.05$ vs. CCS/VEH, +, $p < 0.05$ vs ECS/VEH).

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298 Role of mechanotransduction in CXCL1 secretion from macrophages

299 Resident muscularis macrophages are thought to secrete inflammatory mediators during the
300 development of ileus, and the macrophages are activated by gut manipulation.[26] Therefore, we
301 determined if increased cyclical stretch also increased CXCL1 secretion from macrophages. We used
302 PMA-activated THP1 cells as a model of macrophages. As shown in Figure 6A, increased cyclical
303 stretch (ECS) significantly increases the secretion of CXCL1 from activated THP1 cells ($p < 0.05$ CCS
304 VHE vs. ECS VEH). Similar to hISMCs, inhibition of PAK1 with IPA-3 attenuated the stretch-
305 induced increases in CXCL1 secretion from activated THP1 cells after subjecting cells to ECS ($p <$
306 0,005 ECS VEH vs. ECS IPA-3), but CXCL1 secretion in cells subjected to CCS was not affected by
307 PAK1 inhibition ($p = 0.64$ CCS VEH vs. CCS IPA-3) (Figure 6A). Inhibition of NF-kB with IMD
308 0354 (10 μM) also prevented stretch-induced increases in CXCL1 secretion, similar to hISMC (Figure
309 6B).

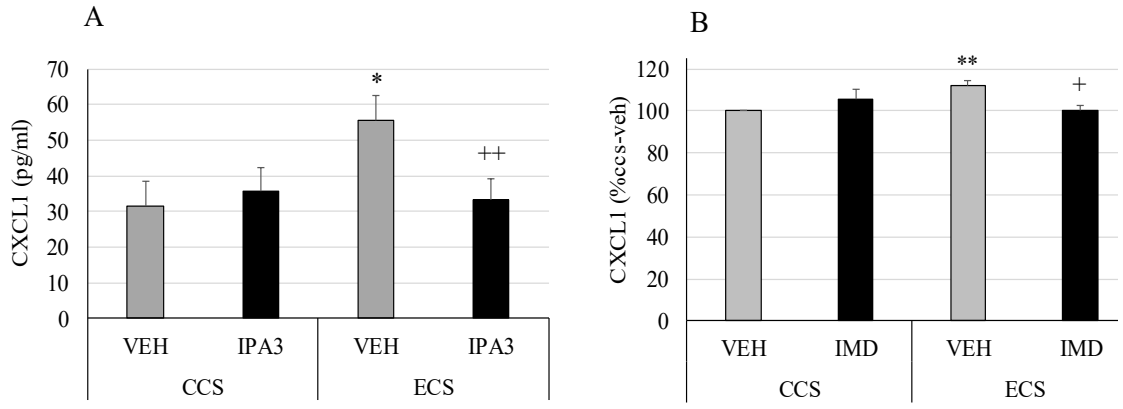


Figure 6. CXCL1 secretion in macrophages. THP1 cells were treated with PMA (100 ng/mL) for 48 h to differentiate into macrophage-like cells. A. CXCL1 secretion in the presence or absence of the PAK1 inhibitor IPA-3 (10 μM) or vehicle (VEH, 0.02% DMSO) and CCS or ECS for 4 hours. CXCL1 was measured in the media. (n = 6 per group; *, $p < 0.05$ vs. CCS/VEH; ++ < 0.005 vs. ECS/VEH). B. CXCL1 secretion in the presence or absence of the NF-kB inhibitor IMD 0354 (10 μM) or vehicle (VE 0.02% DMSO) and CCS or ECS for 4 hours. CXCL1 was measured in the media. (n = 4 per group; **, $p < 0.005$ vs. CCS/VEH; · $p < 0.005$ vs. ECS/VEH)

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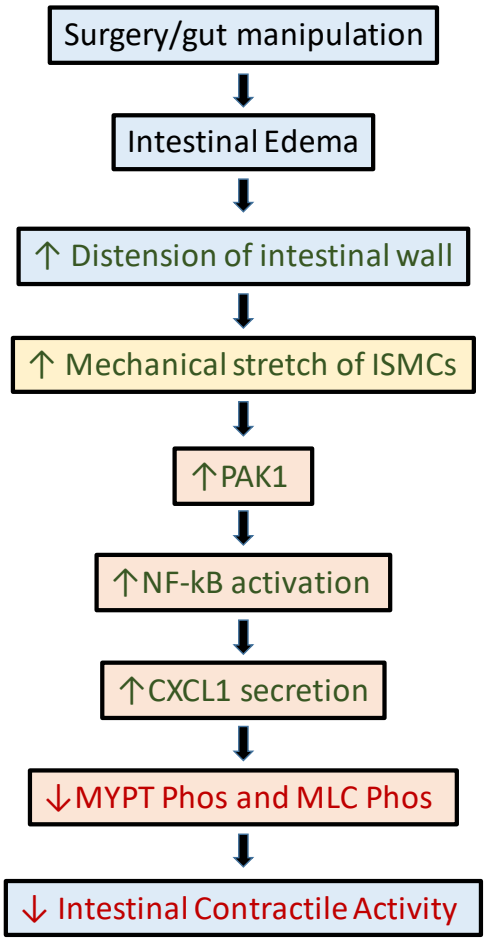


Figure 7. Schematic of the mechanism for decreased intestinal contractile activity after gut manipulation or surgery. Gut manipulation causes edema development, which swells the tissue, leading to increased stretch of cells in the intestinal wall, including intestinal smooth muscle cells (ISMCs) and resident macrophages. The mechanical signal leads to PAK1 activation and, subsequently, NFkB activation. NFkB activation induces CXCL1 secretion from the ISMC and macrophages (and perhaps other cell types). CXCL1 decreases MLC and MYPT1 phosphorylation levels, causing decreased intestinal contractile activity. Organ level changes are indicated by blue boxes, cellular level changes are indicated by a yellow box, and molecular level changes are indicated by orange. Increases are indicated by green text and decreases are indicated by red text.

Discussion

Our results demonstrate that CXCL1, PAK1, and mechanotransduction interact and contribute to the development of ileus. Based on the results of the present study and our previous work, the proposed mechanism for decreased intestinal contractile activity after surgical manipulation of the gut, involving the interactions between CXCL1, PAK1, and mechanotransduction, is shown in Figure 7. Surgical manipulation of the gut causes intestinal interstitial edema (Figure 1F and [17]), which increases mechanical stretch of the intestinal smooth muscle cells and other cells in the wall of the small intestine. Increased stretch of the intestinal wall during the development of intestinal interstitial edema is supported by several previous studies.[21, 22] We previously showed that intestinal edema and increased mechanical stretch of hISMC induced increased PAK1 activity and CXCL1 secretion.[16] [17] In this study, we demonstrated that PAK1 knockdown prevented the induction of CXCL1 in intestinal smooth muscle tissue (Figure 3), supporting the role of PAK1 in inducing CXCL1 secretion.

Furthermore, increased stretch induced NF-kB activity and CXCL1 secretion in hISMC, and PAK1 inhibition prevented the induction of NF-kB activity and CXCL1 (Figure 5). Thus, NF-kB activity and CXCL1 secretion are PAK1-dependent in hISMC. CXCL1 secretion from hISMC and macrophages is dependent on NF-kB activation (Figures 5 and 6). Thus, PAK1 induces NF-kB, which leads to increased CXCL1 secretion. PAK1 modulation of NF-kB activity is supported by multiple studies.[27-29] Finally, increased CXCL1 secretion decreases MLC and MYPT1 phosphorylation (Figure 4), which leads to decreased spontaneous contractile activity [30-32]. Taken together, these data support the proposed mechanism for the involvement of PAK1, CXCL1, and mechanotransduction in the development of ileus, as shown in Figure 7. Edema induces PAK1 activity via mechanotransduction. Increased PAK1 activity increases NF-kB signaling, leading to increased CXCL1 secretion. CXCL1 inhibits MLC and MYPT1 phosphorylation, leading to decreased intestinal contractile activity, resulting in ileus.

In a previous study, CXCL1 increased very early in a gut manipulation model of ileus before increases in other inflammatory mediators, such as IL-1β, and CXCL1 also increased in the plasma of trauma patients who develop ileus.[17] CXCL1, added to the organ bath, inhibits contractile activity.[17] In this study, CXCL1 levels in the intestinal smooth muscle were increased in the wild-type mice but not the PAK1 knockout mice after gut manipulation (Figure 3A and B), despite the development of inflammation (Fig. 1E and F). Of note, CXCL1 is also induced and associated with changes in motility in inflammatory bowel disease and irritable bowel syndrome.[33] Thus, CXCL1, which may be induced by mechanical signals such as bowel distension, is associated with decreased intestinal contractile activity and may be involved in intestinal motility disorders in other diseases.

To understand the changes in intestinal contractile activity after gut manipulation, we calculated not only the integral, which is commonly reported as intestinal contractile activity, but also the integral from the minimum, the tone, which was calculated as the average minimum of the cycle, the contraction amplitude, and the maximum carbachol-induced contractile activity. No differences in contractile activity (integral from 0) were detected after gut manipulation (Figure 2A). In contrast, contractile activity, measured as the integral from the minimum, was significantly reduced after gut

manipulation in the wild-type mice but not the knockout mice (Figure 2B). This difference was due to reduced contraction amplitude in the wildtype but not the PAK1 knockout mice (Figure 2D). The maximum carbachol-induced contractile activity was significantly reduced after gut manipulation regardless of the genotype (Figure 2E). These results demonstrate the inhibitory effects of increased PAK1 activity on intestinal contractile activity, leading to the development of ileus. In addition, these results demonstrate the necessity of utilizing more than the integral when investigating intestinal contractile activity. The differences in contractile activity would not have been detected if only the integral were calculated.

Based on our results, PAK1 knockdown prevented the inhibitory effects of gut manipulation on spontaneous contractile activity, including contraction amplitude (Figure 2D), but not agonist-induced contractile activity (Figure 2E). The western blot results shown in Figure 4 align with these effects of PAK1 knockdown on intestinal contractile activity. Spontaneous contractile activity is mainly myogenic, and agonist-induced contractile activity is regulated via neurohumoral mechanisms. PAK1 knockdown prevented the increases in CXCL1 induced by gut manipulation. Thus, CXCL1 was only increased in the intestinal smooth muscle of the wild-type mice, not the PAK1 knockout mice (Figure 3). CXCL1 treatment of hISMC suppressed myosin light chain and MYPT1 phosphorylation, which regulate myogenic tone, affecting contraction amplitude (Figure 4). However, increased stretch, but not CXCL1, affected M2/M3 muscarinic receptor expression, which regulates response to agonists such as carbachol (Figure 4E–G); this result correlates with Figure 2E, showing decreased agonist-induced contractile activity after gut manipulation (which induced edema and, hence, mechanical signals) and was unaffected by PAK1 knockdown. No significant differences in contraction frequency, which is mainly set by the interstitial cells of Cajal, were detected after gut manipulation or between genotypes. These results indicate that CXCL1 directly affects smooth muscle contraction via regulation of MLC phosphorylation rather than muscarinic receptor signaling.

To understand the effects of PAK1 knockout on inflammation in the ileus model, we measured a panel of cytokines in wild-type and PAK1 knockout mouse intestinal smooth muscle after sham surgery or gut manipulation (Figure 3A and B). Although intestinal edema in the absence of hemodynamic

changes and increased mast cell protease activity indicated that inflammation developed in both the wild-type and PAK1 knockout mice after gut manipulation, fewer cytokines were upregulated in the intestinal smooth muscle of PAK1 knockout mice compared with the wild-type mice after gut manipulation, and several cytokines were differentially regulated, including CXCL1. PAK1 induces the activation and nuclear translocation of NF-κB to regulate a variety of cytokines, including TNF-α, IL-1β, IFN-γ, and CXCL1. [27, 28] Although TNF- α was not upregulated in the ileus mouse model, IL-1β, IFN-γ, and CXCL1 were upregulated in wild-type mice but not PAK1 knockout mice after gut manipulation, suggesting that the upregulation of these cytokines after gut manipulation is mediated by PAK1. The upregulation of NF-kB by mechanical stretch has been shown in previous studies.[34, 35] Our cell experiments (Figure 5) demonstrated that stretch-induced CXCL1 secretion is dependent on NF-kB activity, which is regulated upstream by increased stretch-induced PAK1. Thus, preventing PAK1 activation before surgical manipulation of the gut may prevent post-operative ileus. Unfortunately, PAK1 inhibition may not attenuate ileus once it develops due to the downstream signaling caused by mechanotransductive mechanisms after edema formation. Thus, PAK1 inhibition may be a feasible pretreatment to prevent postoperative ileus, but may not a viable treatment for trauma or critically ill patients who cannot be pretreated.

Ileus is thought to develop in two phases, an early neurogenic phase (within the first few hours after surgery or trauma) and a later (3–4 h) immune-mediated phase.[36] The inflammatory responses during the second phase may be triggered by macrophage activation in the intestinal wall in response to gut manipulation (e.g., during surgery) or exposure to bacterial products.[36] However, in a previous study, CXCL1 increased within 1 hour after gut manipulation in an ileus model, before most inflammatory cytokines increased; IL-1β and TNF-α only increased 4–12 hours after gut manipulation in a gut manipulation model.[17] Thus, it is doubtful that CXCL1 was increased due to the release of inflammatory mediators by macrophage activation and/or neutrophil infiltration. Furthermore, increased cyclical stretch of both cultured intestinal smooth muscle cells and macrophages triggers the increased secretion of CXCL1 (Figures 5 and 6). Edema also develops within the first few hours after gut manipulation, resulting in increased stretch of the intestinal wall.[17] In addition, gut manipulation

may induce mechanotransductive mechanisms. Therefore, CXCL1 is likely increased due to mechanotransduction mechanisms rather macrophage activation/neutrophil infiltration during the development of ileus. The question remains, why does edema develop so quickly, before most inflammatory mediators are released? We speculate that lymphatic vessels that drain the lymph may be disrupted by mechanical stimulation or that mechanical signals themselves trigger edema formation, perhaps before macrophage activation and neutrophil infiltration. Of note, hISMCs subjected to increased cyclical stretch (ECS) responded differently to CXCL1 treatment compared with hISMCs subjected to CCS (Figure 4). Therefore, other signaling mechanisms activated by increased mechanical stretch may contribute to changes in MLC and MYPT1 phosphorylation.

Increased stretch induces CXCL1 secretion in both hISMCs and macrophages in a PAK1 and NF-kB-dependent manner (Figures 5 and 6). In addition to hISMCs and macrophages, CXCL1 expression and secretion are induced by mechanotransduction in a variety of other cell types. For example, CXCL1 expression is induced in liver sinusoidal endothelial cells by cyclical stretch and portal hypertension.[37] Cyclical stretch also induces CXCL1 expression in vascular smooth muscle cells.[38] CXCL1 is induced in hypertrophic cardiomyocytes,[39] and applying mechanical stress to bone fibroblasts induces the expression of CXCL1.[40] Although it is unclear if there is a common pathway for stretch-induced CXCL1 expression, the signaling pathways activated by stretch may converge. The CXCL1 promoter has binding sites for NF-kB and Sp1 that increase CXCL1 expression and STAT1 and CDP sites that decrease CXCL1 expression.[41, 42] Mechanical stretch can activate integrin and Piezo channels that stimulate SP1 and possibly induce CXCL1 expression.[37] The roles of these alternative pathways in hISMCs are unclear.

In conclusion, our results indicate the involvement of mechanotransduction, CXCL1, and PAK1 in the development of ileus. The suppressed spontaneous intestinal contractile activity induced by gut manipulation was attenuated in the PAK1 knockout mouse model. Although inflammation likely developed in the PAK1 knockout mice, as indicated by the mast cell protease activity and edema, the upregulation of several cytokines, including CXCL1, was attenuated. We previously showed that PAK1 was induced by increased cyclical stretch and edema; therefore, we investigated the effects of

increased cyclical stretch on CXCL1 secretion. Our results show that increased cyclical stretch increased CXCL1 secretion in a PAK1 and NF-kB-dependent manner. Although pretreatment of surgical patients with a PAK1 inhibitor may prevent the development of ileus in surgical patients, the off-target toxicity of most PAK1 inhibitors precludes their use for the treatment of ileus.[43] However, understanding the mechanisms for the development of ileus will help identify potential downstream targets for treating ileus, such as CXCR2 antagonists to block CXCL1 signaling.

Abbreviations

CCS	control cyclical stretch
CXCL1	C-X-C motif chemokine ligand 1
ECS	edema cyclical stretch
GM	gut manipulation
HISMCs	Human intestinal smooth muscle cells
MCP-1	monocyte chemoattractant protein-1
MLC	myosin light chain
MYPT1	myosin phosphatase target subunit
NK-κB	Nuclear factor (NF)-κB
PAK1	P21-activated kinase
TIMP1	Tissue Inhibitor of Metalloproteinases 1

Ethics Approval

All animal experiments were approved by the University of Texas Health Science Center Animal Welfare Committee (Protocol number HSC-AWC-13-090) and the Animal Welfare Committee of the University of Debrecen (DEMAB/44-1/2025). All experiments complied with the NIH 'Guide for the Care and Use of Laboratory Animals.'

Consent for publication

Not applicable.

Data availability

The data in this manuscript are available at https://doi.org/10.6084/m9.figshare.32880464.

Conflict of Interest Statement

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Grant Support

Hungarian National Research, Development, and Innovation Office (NKFI 120669) and Zoltan Szinte via the Foundation for the Oncology Patients of Debrecen

Authors' Contributions

Conceptualization: KU, CSC; Methodology: KU, JBK, TD; Validation: KU; Formal analysis: KU; Investigation: JBK, AK, TD, DB, KU; Resources: KU, CSC; Data Curation: KU; Drafting-Original Draft- KU; Drafting- Review and Editing: JBK, AK, TD, DB, CSC, KU; Visualization: JBK, AK, KU; Supervision: KU; Funding acquisition: KU, CSC.

Acknowledgements

Not applicable.

References

1. Aries P, Huet O. Ileus in the critically ill: causes, treatment and prevention. Minerva Anestesiol 2020;86:974-983; doi: 10.23736/S0375-9393.20.14778-3
2. Virani FR, Peery T, Rivas O et al. Incidence and Effects of Feeding Intolerance in Trauma Patients. JPEN J Parenter Enteral Nutr 2019;43:742-749; doi: 10.1002/jpen.1469
3. Venara A, Neunlist M, Slim K et al. Postoperative ileus: Pathophysiology, incidence, and prevention. J Visc Surg 2016;153:439-446; doi: 10.1016/j.jviscsurg.2016.08.010
4. Nguyen NQ, Besanko LK, Burgstad C et al. Delayed enteral feeding impairs intestinal carbohydrate absorption in critically ill patients. Crit Care Med 2012;40:50-54; doi: 10.1097/CCM.0b013e31822d71a6
5. Reignier J, Darmon M, Sonneville R et al. Impact of early nutrition and feeding route on outcomes of mechanically ventilated patients with shock: a post hoc marginal structural model study. Intensive Care Med 2015;41:875-886; doi: 10.1007/s00134-015-3730-4
6. Choi YK, Kim HJ, Ahn J, Ryu JA. Impact of early nutrition and feeding route on clinical outcomes of neurocritically ill patients. PLoS One 2023;18:e0283593; doi: 10.1371/journal.pone.0283593
7. Talebi S, Zeraattalab-Motlagh S, Vajdi M et al. Early vs delayed enteral nutrition or parenteral nutrition in hospitalized patients: An umbrella review of systematic reviews and meta-analyses of randomized trials. Nutr Clin Pract 2023;38:564-579; doi: 10.1002/ncp.10976
8. Peters EG, Pattamatta M, Smeets BJJ et al. The clinical and economical impact of postoperative ileus in patients undergoing colorectal surgery. Neurogastroenterol Motil 2020;32:e13862; doi: 10.1111/nmo.13862

9. Senagore AJ. Pathogenesis and clinical and economic consequences of postoperative ileus. Clin Exp Gastroenterol 2010;3:87-89; doi:
10. Asgeirsson T, El-Badawi KI, Mahmood A et al. Postoperative ileus: it costs more than you expect. J Am Coll Surg 2010;210:228-231; doi: 10.1016/j.jamcollsurg.2009.09.028
11. Traeger L, Koullouros M, Bedrikovetski S et al. Cost of postoperative ileus following colorectal surgery: A cost analysis in the Australian public hospital setting. Colorectal Dis 2022;24:1416-1426; doi: 10.1111/codi.16235
12. Traeger L, Koullouros M, Bedrikovetski S et al. Global cost of postoperative ileus following abdominal surgery: meta-analysis. BJS Open 2023;7: doi: 10.1093/bjsopen/zrad054
13. Livingston EH, Passaro EP, Jr. Postoperative ileus. Dig Dis Sci 1990;35:121-132; doi:
14. Barletta JF, Senagore AJ. Reducing the burden of postoperative ileus: evaluating and implementing an evidence-based strategy. World J Surg 2014;38:1966-1977; doi: 10.1007/s00268-014-2506-2
15. Traut U, Brugger L, Kunz R et al. Systemic prokinetic pharmacologic treatment for postoperative adynamic ileus following abdominal surgery in adults. Cochrane Database Syst Rev 2008;CD004930; doi: 10.1002/14651858.CD004930.pub3
16. Chu J, Pham NT, Olate N et al. Biphasic regulation of myosin light chain phosphorylation by p21-activated kinase modulates intestinal smooth muscle contractility. J Biol Chem 2013;288:1200-1213; doi: 10.1074/jbc.M112.370718
17. Docsa T, Bhattarai D, Sipos A et al. CXCL1 is upregulated during the development of ileus resulting in decreased intestinal contractile activity. Neurogastroenterol Motil 2020;32:e13757; doi: 10.1111/nmo.13757
18. Kalff JC, Schraut WH, Simmons RL, Bauer AJ. Surgical manipulation of the gut elicits an intestinal muscularis inflammatory response resulting in postsurgical ileus. Ann Surg 1998;228:652-663; doi: 10.1097/00000658-199811000-00004
19. Schneider CA, Rasband, W. S., Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 2012;9:671-675; doi:
20. Girish V, Vijayalakshmi A. Affordable image analysis using NIH Image/ImageJ. Indian J Cancer 2004;41:47; doi:
21. Cox CS, Jr., Radhakrishnan R, Villarrubia L et al. Hypertonic saline modulation of intestinal tissue stress and fluid balance. Shock 2008;29:598-602; doi: 10.1097/SHK.0b013e318157eba7
22. Young J, Riviere B, Cox CS, Jr., Uray K. A mathematical model of intestinal oedema formation. Math Med Biol 2014;31:1-15; doi: 10.1093/imammb/dqs025
23. Inoh H, Ishiguro N, Sawazaki S et al. Uni-axial cyclic stretch induces the activation of transcription factor nuclear factor kappaB in human fibroblast cells. FASEB J 2002;16:405-407; doi: 10.1096/fj.01-0354fje
24. Leychenko A, Konorev E, Jijiwa M, Matter ML. Stretch-induced hypertrophy activates NFkB-mediated VEGF secretion in adult cardiomyocytes. PLoS One 2011;6:e29055; doi: 10.1371/journal.pone.0029055
25. Amiri KI, Richmond A. Fine tuning the transcriptional regulation of the CXCL1 chemokine. Prog Nucleic Acid Res Mol Biol 2003;74:1-36; doi: 10.1016/s0079-6603(03)01009-2
26. Wehner S, Behrendt FF, Lyutenski BN et al. Inhibition of macrophage function prevents intestinal inflammation and postoperative ileus in rodents. Gut 2007;56:176-185; doi: 10.1136/gut.2005.089615
27. Dammann K, Khare V, Lang M et al. PAK1 modulates a PPARgamma/NF-kappaB cascade in intestinal inflammation. Biochim Biophys Acta 2015;1853:2349-2360; doi: 10.1016/j.bbamcr.2015.05.031

28. Frost JA, Swantek JL, Stippec S et al. Stimulation of NFkappa B activity by multiple signaling pathways requires PAK1. J Biol Chem 2000;275:19693-19699; doi: 10.1074/jbc.M909860199
29. Foryst-Ludwig A, Naumann M. p21-activated kinase 1 activates the nuclear factor kappa B (NF-kappa B)-inducing kinase-Ikappa B kinases NF-kappa B pathway and proinflammatory cytokines in Helicobacter pylori infection. J Biol Chem 2000;275:39779-39785; doi: 10.1074/jbc.M007617200
30. Chu J, Miller CT, Kislitsyna K et al. Decreased myosin phosphatase target subunit 1(MYPT1) phosphorylation via attenuated rho kinase and zipper-interacting kinase activities in edematous intestinal smooth muscle. Neurogastroenterol Motil 2012;24:257-266, e109; doi: 10.1111/j.1365-2982.2011.01855.x
31. Perrino BA. Calcium Sensitization Mechanisms in Gastrointestinal Smooth Muscles. J Neurogastroenterol Motil 2016;22:213-225; doi: 10.5056/jnm15186
32. Uray KS, Laine GA, Xue H et al. Intestinal edema decreases intestinal contractile activity via decreased myosin light chain phosphorylation. Crit Care Med 2006;34:2630-2637; doi: 10.1097/01.CCM.0000239195.06781.8C
33. Korbecki J, Szatkowska I, Kupnicka P et al. The Importance of CXCL1 in the Physiological State and in Noncancer Diseases of the Oral Cavity and Abdominal Organs. Int J Mol Sci 2022;23: doi: 10.3390/ijms23137151
34. Tu PC, Pan YL, Liang ZQ et al. Mechanical Stretch Promotes Macrophage Polarization and Inflammation via the RhoA-ROCK/NF-kappaB Pathway. Biomed Res Int 2022;2022:6871269; doi: 10.1155/2022/6871269
35. Zhao H, Hiroi T, Hansen BS, Rade JJ. Cyclic stretch induces cyclooxygenase-2 gene expression in vascular endothelial cells via activation of nuclear factor kappa-beta. Biochem Biophys Res Commun 2009;389:599-601; doi: 10.1016/j.bbrc.2009.09.028
36. Wang Z, Stakenborg N, Boeckxstaens G. Postoperative ileus-Immune mechanisms and potential therapeutic interventions. Neurogastroenterol Motil 2025;37:e14951; doi: 10.1111/nmo.14951
37. Hilscher MB, Sehrawat T, Arab JP et al. Mechanical Stretch Increases Expression of CXCL1 in Liver Sinusoidal Endothelial Cells to Recruit Neutrophils, Generate Sinusoidal Microthombi, and Promote Portal Hypertension. Gastroenterology 2019;157:193-209 e199; doi: 10.1053/j.gastro.2019.03.013
38. Zhao J, Nishimura Y, Kimura A et al. Chemokines protect vascular smooth muscle cells from cell death induced by cyclic mechanical stretch. Sci Rep 2017;7:16128; doi: 10.1038/s41598-017-15867-8
39. Komuro J, Hashimoto H, Katsuki T et al. Heart failure-specific cardiac fibroblasts contribute to cardiac dysfunction via the MYC-CXCL1-CXCR2 axis. Nat Cardiovasc Res 2025;4:1135-1151; doi: 10.1038/s44161-025-00698-y
40. Man QW, Li RF, Li SR et al. Single-Cell RNA Sequencing Reveals CXCLs Enriched Fibroblasts Within Odontogenic Keratocysts. J Inflamm Res 2021;14:7359-7369; doi: 10.2147/JIR.S342951
41. Korbecki J, Barczak K, Gutowska I et al. CXCL1: Gene, Promoter, Regulation of Expression, mRNA Stability, Regulation of Activity in the Intercellular Space. Int J Mol Sci 2022;23: doi: 10.3390/ijms23020792
42. Nirodi C, Hart J, Dhawan P et al. The role of CDP in the negative regulation of CXCL1 gene expression. J Biol Chem 2001;276:26122-26131; doi: 10.1074/jbc.M102872200
43. Semenova G, Chernoff J. Targeting PAK1. Biochem Soc Trans 2017;45:79-88; doi: 10.1042/BST20160134

Figure Legends

Figure 1. Characterization of PAK1 knockout mice after sham surgery (SHAM) or gut manipulation (GM). A. Western blot showing the expression of PAK1 in wild-type (+/+), heterozygous (+/−), and homozygous (−/−) PAK1 knockout mice. B. Quantification of PAK1 expression 24 hours after SHAM or GM. (n = 2 per group; +, $p < 0.05$ and ++ $p < 0.005$ vs. respective wild-type; *, $p < 0.05$ vs. SHAM in same genotype) C. Body weights in male and female mice from the three mouse genotypes at 3 months of age. (n = 6–24 per group; *, $p < 0.05$ vs. wild-type of same sex; +, $p < 0.05$ vs. female). D. Small intestinal lengths 24 hours after SHAM or GM. (n = 4 per group; *, $p < 0.05$ vs. SHAM in same genotype group). E. Mast cell protease levels in the peritoneal fluid 4 hours after SHAM or GM. (n = 6 per group; *, $p < 0.05$ and ** $p < 0.005$ vs. SHAM in same genotype group). F. Wet-to-dry weight ratios, indicating edema development, 24 hours after SHAM or GM. (** $p < 0.005$ vs. SHAM in same genotype group).

Figure 2. Contractile activity 24 hours after gut manipulation or sham surgery in wild-type, heterozygous, and homozygous PAK1 knockout mice. All contractile activity values reflect the averages over 4 minutes of data. A. Contractile activity, calculated as the integral (from zero). B. Contractile activity calculated as the integral from the minimum value. C. Intestinal tone, calculated as the average minimum value. D. Contraction amplitude, calculated as the average contraction cycle amplitude. E. Maximum agonist-induced contractile activity calculated as the maximum area under the curve after step-wise increased carbachol treatment from 10^{-9}–10^{-4}. (n = 11–12 in wild-type groups, 12–13 in heterozygote groups, and 5 in homozygote groups; *, $p < 0.05$ vs. respective SHAM in same genotype group; +, $p < 0.05$ vs. wildtype in same treatment group; ǂ, $p < 0.05$ vs. heterozygotes in same treatment group). F. Contraction frequency.

Figure 3. Changes in inflammatory mediators in intestinal smooth muscle tissue 4 hours after gut manipulation or sham surgery in wildtype, heterozygous, and homozygous PAK1 knockout mice. A, B. The ratios of cytokines after sham surgery (laparotomy only, SHAM) versus gut manipulation (GM) were measured using cytokine arrays in wild-type and PAK1 knockout mice, respectively (n = 3 per group). C–D. Changes in MCP-1, CXCL1, and IL1b levels, respectively, after sham surgery (laparotomy only, SHAM) or gut manipulation (GM) in intestinal smooth muscle tissue from wild-type and PAK1 knockout mice. Cytokines were measured using ELISAs. (n = 3–11 per group; *, $p < 0.05$ vs. SHAM).

Figure 4. Changes in myosin light chain (MLC) and MYPT1 phosphorylation and muscarinic receptor levels in primary human intestinal smooth muscle cells (hISMC) after treatment with CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours. A–B. Representative western blots and quantification of changes in the ratio of phospho-MLC to total MLC in response to treatment with varying concentrations of CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours. (n = 2–7 per group; *, $p < 0.05$ vs. CCS; ***, $p < 0.0001$ vs. CCS). C–D. Representative western blots and quantification of changes in phospho-MYPT1 (P696) to total MYPT1 in primary hISMC in response to treatment with 100 ng/ml CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours (n = 5 per group; *, $p < 0.05$ vs. CCS). E–G. Representative western blots and quantification of changes in M2 and M3 muscarinic receptors in primary hISMC in response to treatment with 200 ng/ml CXCL1 and subjecting cells to CCS or ECS stretching protocols for 4 hours (n = 3 per group; *, $p < 0.05$ vs. CCS).

Figure 5. The regulation of CXCL1 secretion from human intestinal smooth muscle cells (hISMC). A. CXCL1 secretion after subjecting hISMCs to CCS or ECS stretching protocols. (n = 6 per group; * $p < 0.05$ vs. CCS). B–C. CXCL1 secretion in the presence or absence of the PAK1 inhibitor IPA-3 (10 μM) and subjecting hISMCs to CCS or ECS. (n = 8 per group; ** $p < 0.05$ vs. VEH). D. NF-kB

activity in the presence or absence of IPA-3 and subjecting hISMCs to CCS or ECS. (n = 5 per group; ** $p < 0.05$ vs. CCS/DMSO). E. CXCL1 secretion in the presence or absence of the NF-kB inhibitor IMD (1.5 μM) and subjecting cells to CCS or ECS. (n = 3 per group; * $p < 0.05$ vs. CCS/VEH, +, $p < 0.05$ vs ECS/VEH).

Figure 6. CXCL1 secretion in macrophages. THP1 cells were treated with PMA (100 ng/mL) for 48 h to differentiate into macrophage-like cells. A. CXCL1 secretion in the presence or absence of the PAK1 inhibitor IPA-3 (10 μM) or vehicle (VEH, 0.02% DMSO) and CCS or ECS for 4 hours. CXCL1 was measured in the media. (n = 6 per group; *, $p < 0.05$ vs. CCS/VEH; ++, $p < 0.005$ vs. ECS/VEH). B. CXCL1 secretion in the presence or absence of the NF-kB inhibitor IMD 0354 (10 μM) or vehicle (VEH, 0.02% DMSO) and CCS or ECS for 4 hours. CXCL1 was measured in the media. (n = 4 per group; **, $p < 0.005$ vs. CCS/VEH; +, $p < 0.005$ vs. ECS/VEH)

Figure 7. Schematic of the mechanism for decreased intestinal contractile activity after gut manipulation or surgery. Gut manipulation causes edema development, which swells the tissue, leading to increased stretch of cells in the intestinal wall, including intestinal smooth muscle cells (ISMCs) and resident macrophages. The mechanical signal leads to PAK1 activation and, subsequently, NFkB activation. NFkB activation induces CXCL1 secretion from the ISMC and macrophages (and perhaps other cell types). CXCL1 decreases MLC and MYPT1 phosphorylation levels, causing decreased intestinal contractile activity. Organ-level changes are indicated by blue boxes, cellular-level changes are indicated by a yellow box, and molecular-level changes are indicated by orange. Increases are indicated by green text, and decreases are indicated by red text.

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