

Inflammation and Impaired Gut Physiology in Post-operative Ileus: Mechanisms and the Treatment Options

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Post-operative ileus (POI) is the transient cessation of coordinated gastrointestinal motility after abdominal surgical intervention. It decreases quality of life, prolongs length of hospital stay, and increases socioeconomic costs. The mechanism of POI is complex and multifactorial, and has been broadly categorized into neurogenic and inflammatory phase. Neurogenic phase mediated release of corticotropin-releasing factor (CRF) plays a central role in neuroinflammation, and affects both central autonomic response as well hypothalamic-pituitary-adrenal (HPA) axis. HPA-stress axis associated cortisol release adversely affects gut microbiota and permeability. Peripheral CRF (ρ CRF) is a key player in stress induced gastric emptying and colonic transit. It functions as a local effector and interacts with the CRF receptors on the mast cell to release chemical mediators of inflammation. Mast cells proteases disrupt epithelial barrier via protease activated receptor-2 (PAR-2). PAR-2 facilitates cytoskeleton contraction to reorient tight junction proteins such as occludin, claudins, junctional adhesion molecule, and zonula occludens-1 to open epithelial barrier junctions. Barrier opening affects the selectivity, and hence permeation of luminal antigens and solutes in the gastrointestinal tract. Translocation of luminal antigens perturbs mucosal immune system to further exacerbate inflammation. Stress induced dysbiosis and decrease in production of short chain fatty acids add to the inflammatory response and barrier disintegration. This review discusses potential mechanisms and factors involved in the pathophysiology of POI with special reference to inflammation and interlinked events such as epithelial barrier dysfunction and dysbiosis. Based on this review, we recommend CRF, mast cells, macrophages, and microbiota could be targeted concurrently for efficient POI management.

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Key Words

Ileus; Inflammation mediators; Macrophages; Mast cells; Permeability

Introduction

Post-operative ileus (POI) is the transient cessation of coordinated gastrointestinal (GI) motility after abdominal surgical intervention.¹⁻³ It leads to various symptoms such as nausea, vomit-

ing, abdominal discomfort, and inability to pass stools or tolerate a solid diet. In addition to these symptoms, POI is associated with decreased quality of life, prolonged length of stay in a hospital, and increased socioeconomic costs and decreased patients' satisfaction with surgery.¹⁻⁴ "Uncomplicated" or "normal" POI is an unavoidable process which generally resolves within 3 days, while

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“prolonged” or “paralytic” post-operative ileus extends beyond the duration of 3 days.⁵⁻⁷ The mechanism of POI is suggested to be complex and multifactorial, and has been broadly categorized into neurogenic and inflammatory phase.⁸⁻¹⁰ The neurogenic phase is short and ends early after the surgery. The second is the inflammatory phase, which starts 3-4 hours after surgery and persists much longer.^{11,12} Therefore, this review highlights the factors that induce inflammation and how it impairs gut physiology in the POI. It will provide an opportunity and direction for effective management and treatments options to ease the burden of POI.

Neuroinflammation and Barrier Dysfunction —

Neural pathways are complicated, and are influenced by the number of factors including intensity of the stimulus or stress.^{1,11,12} Briefly, incision of the skin and laparotomy induce the activation of inhibitory spinal and sympathetic reflexes or the adrenergic inhibitory pathway.^{1,2,12} Furthermore, the handling of the bowel stimulates supra-spinal pathway to activate the hypothalamic–pituitary–adrenal (HPA) axis.^{1,2,12} The sensory afferent neurons of this pathway then send signals to the central nervous system to release stress neuropeptides, such as substance P, calcitonin-

gene related peptide (CGRP), and corticotropin-releasing factor (CRF).¹¹⁻¹⁴ Several studies reported activation of CRF neurons and CRF release in the brain due to the abdominal surgery.^{11,12,15,16} The POI model of our study corroborate these findings, as CRF expression in the hypothalamus increased significantly compared to the control group.¹² The release of CRF is considered to be a key molecule in neuroinflammation (Fig. 1).^{1,11,12,15-18} This leads to de novo synthesis of central proinflammatory cytokines such IL-1 β , IL-6, and TNF- α .^{19,20} CRF triggers stress pathways through receptors, CRF receptor type 1 (CRF1) and type 2.^{21,22} The central CRF is expressed predominantly in the paraventricular nucleus of the hypothalamus,^{23,24} and is involved in alteration of autonomic nervous system activity in the brain to secrete catecholamine to diminish gastric vagal efferent activity and inhibit GI motility.^{25,26} Several studies reported a parallel between central CRF mediated autonomic response as well its ability to activate the HPA-stress-axis.^{27,28} Lenz et al,²⁷ showed the independence of these 2 pathways in hypophysectomized rats. CRF release activates the HPA axis through pituitary CRF1 receptor that leads to secretion of adrenocorticotropic hormone (ACTH).^{27,28} Similarly, the level of ACTH in POI model of our study was significantly increased compared to healthy controls.¹² In addition, the higher level of ACTH was

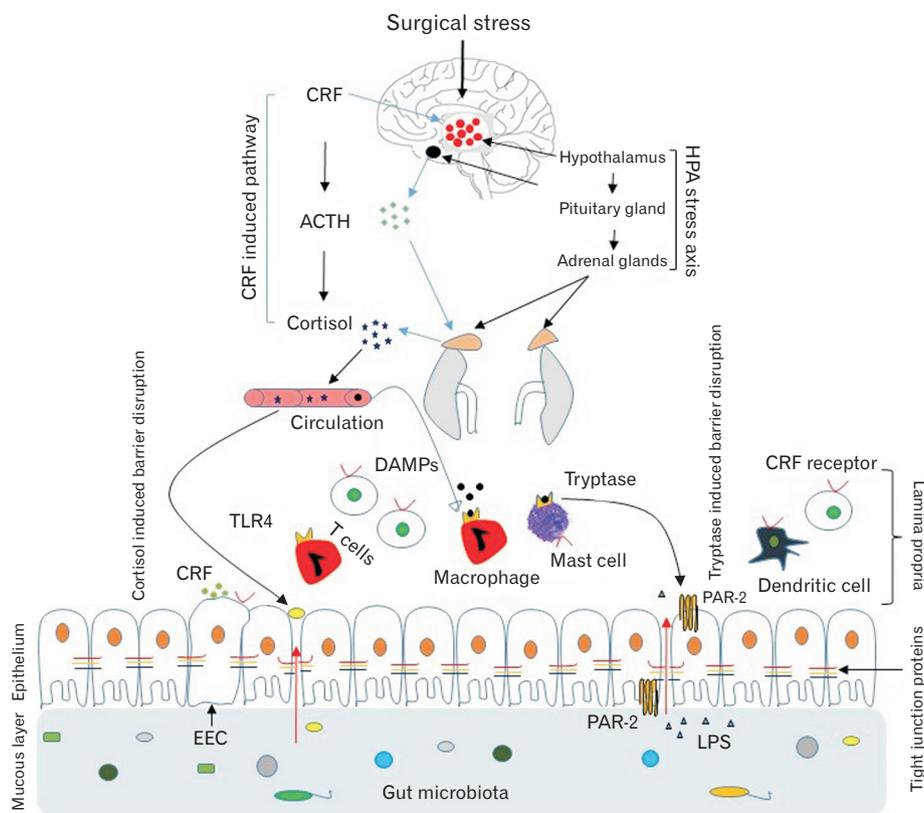


Figure 1. Schematic illustration of stress–corticotropin-releasing factor (CRF) induced neuroinflammation and barrier disruption with focus on the activation of hypothalamus–pituitary–adrenal (HPA) axis. HPA axis mediated production of cortisol alter gut microbiota, increase lipopolysaccharide (LPS), and impairs gut barrier via reorientation of tight junction proteins. Microbiota alteration also affects its metabolites, ie, short-chain fatty acids to modify epithelial barrier integrity, exacerbate immune cells activation, and add to psychiatric morbidities. ACTH, adrenocorticotropic hormone; DAMPs, damage associated molecular pattern; TLR4, Toll-like receptors-4; PAR-2, protease activated receptor-2; EEC, enteroendocrine cells.

directly linked to the inhibition of GI motility.¹² ACTH further activates the adrenal glands to release high levels of cortisol.²⁷⁻³¹ There is an increasing evidence that shows the involvement of cortisol in dysbiosis and increase in gut permeability.^{28,31} Stress (non-infectious) induced cortisol increased endotoxemia such as lipopolysaccharides (LPS), and it corresponded LPS from the commensal flora.^{28,31,32} Interestingly, stress alone could induce adequate LPS from the commensals to initiate the disruption of the intestinal barrier and increase in gut permeability.^{28,31,32} So a threshold level of CRF or cortisol is enough to initiate the disruption of the intestinal barrier and increase in permeability.³³⁻³⁶ It has been reported that corticoid receptors regulates the expression of tight junction proteins (TJPs) such as claudin-1 and occludin.^{37,38} Additionally, dysbiosis decreases bacterial metabolites, short chain fatty acids (SCFAs) such as butyrate, acetate, and propionate to alter gut barrier integrity.^{28,31,39-42} Therefore, stress induced cortisol not only alters gut microbiota but also impairs barrier integrity via modifying TJPs (Fig. 1). Impaired gut barrier induces bacterial translocation resulting in activation of the local immune system and inflammation, thereby increasing the release of the cytokines.^{11,43,44} Several studies reported CRF mediated increase in the expression of Toll-like receptor 4 (TLR4) on macrophages and high level of inflammatory cytokines production via stress induced LPS.^{43,44}

Peripheral Corticotropin-releasing Factor, Immune Cell Activation, and Epithelial Barrier Dysfunction

Recent studies have shown that CRF ligands and receptors are not only expressed in the brain but also in peripheral organs including GI tract.^{31,45} Peripheral CRF (ρ CRF) is released from cells such as neuronal, enterochromaffin, and immune cells (mast cells) in the lamina propria, submucosa, and muscle layers.⁴⁵⁻⁴⁹ CRF1 are expressed by neurons in both myenteric and submucosal plexuses of the enteric nervous system.⁵⁰ Interestingly, both central and peripheral administration of CRF and its receptor antagonists results in similar gut transit alterations, though mechanisms and sites of action are distinct.⁵¹⁻⁵³ Similarly, we also reported significant increase in GI transit when treated (subcutaneously) with higher doses of selective non-peptide CRF1 antagonist CP-154 526 in a POI model compared to sham controls.¹² Our finding was consistent with previous study that showed the CRF1 antagonist improve delayed gastric emptying induced by laparotomy plus cecal manipulation in mice.⁵⁴ Peripheral treatment with human/rat CRF, an agonist for CRF receptor, modifies the delayed GI transit in our

POI model.¹² In addition, exogenous ρ CRF induced alterations of gastric transit was reversed by CRF receptor blockade by astressin (a non-selective CRF receptor antagonist).³⁵ The data from our study strengthen the notion that CRF acts through ρ CRF pathways, and corroborated earlier findings that ρ CRF is a key player in stress induced gastric emptying and colonic transit.^{35,51-54} Lenz et al²⁷ has shown that the peripherally injected CRF did not activate CRF receptors in the brain. This supports the fact that peripheral release of CRF ligand from afferent nerve terminals and other cells of GI tract is under the influence of autonomic pathways.⁵⁵ Several studies reported close proximity of the afferent nerve terminals to the mast cells in the mesentery and mucosa of GI tract (Fig. 2).⁵⁶⁻⁶² Peripheral CRF functions as a local effector, and it interacts with the CRF receptors present on mast cells.^{31,47,54,63-65} Peripheral CRF-receptor interactions activate mast cells to degranulate and release chemical mediators such as serine proteases (tryptase) and TNF- α to impair epithelial barrier integrity via modifying TJPs.^{33,63} However, epithelial barrier disruption via TNF- α and proteases involves different mechanisms.^{63,66} TNF- α enhances epithelial permeability through increased myosin light-chain kinase (MLCK) expression, activation of nuclear factor kappa B (NF- κ B) pathway, and reorientation and downregulation of TJPs of the gut.⁶⁷ The modified TJPs increase gut permeability, luminal translocation, and immune activation that complements inflammatory cascades.

Mast Cell Protease-mediated Protease Activated Receptor-2 Activation and Barrier Disruption

Neuropeptides such as substance P and CGRP initiate proteases mediated epithelial barrier disruption via protease-activated receptor-2 (PAR-2) signaling mechanisms.⁶⁸⁻⁷⁰ PARs are 7 transmembrane-spanning, G-protein-coupled receptors, activated by the cleavage of their N-terminal domain by proteases such as tryptase or trypsin.⁷¹ PAR-2 can be activated by both endogenous and luminal proteases and it is localized on both basolateral and apical sites of enterocytes and is involved in multiple functions including the maintenance of gut permeability (Fig. 2).^{68,72,73} Activation of either luminal or internal basolateral PAR-2 affects gut paracellular permeability by modulating the degree of cytoskeleton contraction.⁷⁰⁻⁷⁵ We explored the role of PAR-2 in POI stress model, and there was an increase in PAR-2 expression in the POI groups compare to sham in the colon.¹⁰ The increase in PAR-2 expression correlated with enhanced initiation and activation of mast cells in the colon.¹⁰ Though there was marked increase in PAR-2 expression

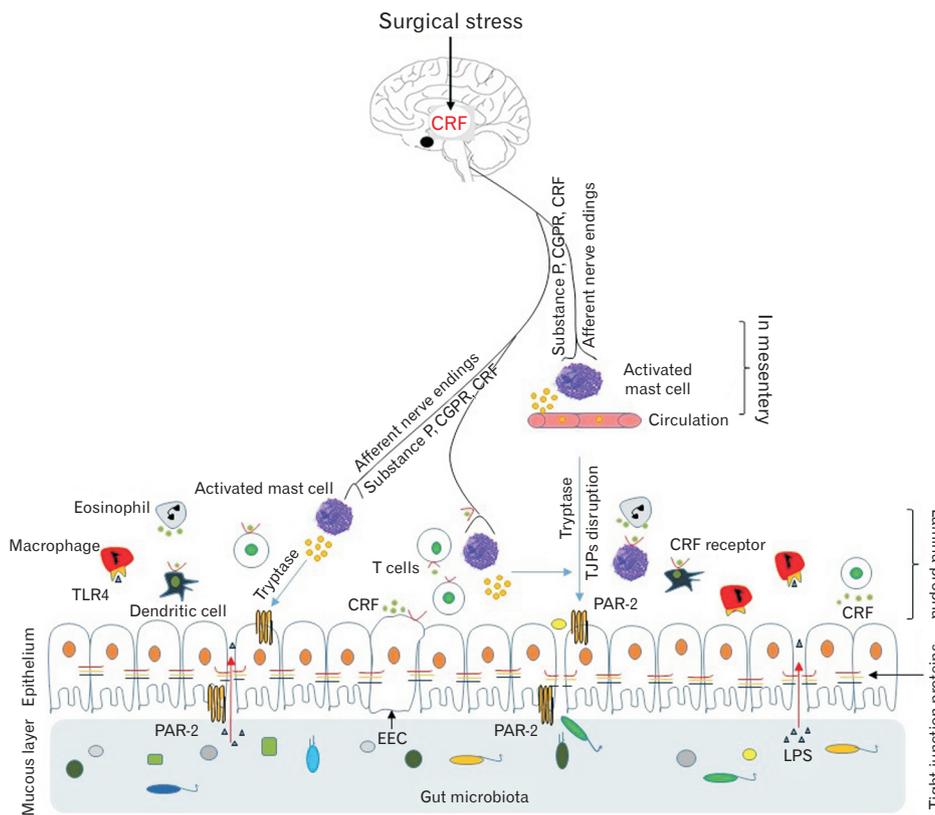


Figure 2. Schematic illustration of autonomic nerve stimulation and increase in gut permeability. The release of stress peptides such as substance P, calcitonin gene-related peptide (CGPR), and corticotropin-releasing factor (CRF) from afferent nerve endings activates mast cells in the mesentery as well as in the mucosal layer. Activated mast cells release chemical mediators such as proteases (tryptase) to modify tight junction proteins (TJPs) and increase intestinal permeability and further activation of immune cells. TLR4, Toll-like receptors 4; PAR-2, protease activated receptor-2; EEC, enteroendocrine cells; LPS, lipopolysaccharide.

in the colon, it failed to prove statistical significance, indicating that other mediators such as histamines, cytokines, and TNF- α operate along with PAR-2 during the genesis of POI.⁶⁷ PAR-2 facilitates cytoskeleton contraction to trigger phosphorylation of myosin light chain catalyzed by the MLCK.⁶⁸ Epithelial cell cytoskeletal contraction re-orient TJPs such as occludin, claudins, junctional adhesion molecule-1, and zonula occludens-1, and opening of the epithelial barrier junction. We also identified the changes in gut paracellular permeability through the expression of claudins, and there was decreased expression of claudin-1 but increased expression of claudin-2 in the POI groups compared to sham.¹⁰ Claudins are classified as either barrier forming or pore forming. Among approximately 24 claudin genes, claudin-1 is known as a barrier-forming protein that decreases paracellular permeability.⁷⁶ On the other hand, claudin-2 is a pore-forming protein that increases paracellular permeability through the formation of channels.⁷⁷ These events affect the selectivity of this pathway and hence permeation of luminal noxious molecules (bacteria, LPS) and solutes in GI tract that induce a perturbation of the mucosal immune system and inflammation.^{68-70,78} LPS is a ligand of TLR4 on macrophages, and its interaction activates TLR4-cytokine signaling and contributes in the inflammatory cascade.^{31,43,78-80}

Dysbiosis, Inflammatory Response, and Barrier Disruption

During metabolism, the host and its gut microbiota coproduce a spectrum of metabolites, such as SCFAs that are essential for health.⁸¹ SCFAs mainly consist of acetate, propionate, and butyrate, and it functions through either G protein coupled receptors (GPCRs) or histone deacetylases.^{82,83} SCFAs are a vital fuel for intestinal epithelial cells and are known to strengthen the gut barrier function.⁸⁴⁻⁸⁷ Butyrate has been demonstrated to play a key role in the maintenance of the intestinal barrier function⁸⁴⁻⁸⁸ and immunomodulation.⁸⁹⁻⁹¹ It also triggers differentiation of colonic regulatory T cells to assist in suppressing inflammatory reactions.^{43,89-91} Surgical interventions which represents one of the physiologic stress are reported to induce a numerical and compositional shift in the gut microbiota.^{19,31,92-94} The relative shift in its composition also affects the bacterial metabolites.⁹²⁻⁹⁴ The POI model of our study clearly reported a distinct change in the microbial community.⁹⁴ The population size of the lactic acid-producing bacteria, including the genera *Bifidobacterium* and *Lactobacillus* decreased, and the population sizes of *Bacteroides* and *Blautia* increased in the POI groups.⁹⁴ Our

study also demonstrated a significant change in the microbial community that reduces the fecal butyrate level.⁹⁴ Apart from the POI stress model, repeated water avoidance stress also decreased occludin expression with reduced butyrate-producing microbiota.^{39,40} Parada Venegas et al⁹⁵ clearly showed significant decrease in butyrate-producing bacteria, especially *Faecalibacterium prausnitzii*, that diminish the SCFAs in feces. Notably, these findings are consistent with previous studies showing that dysbiosis-related decrease in the synthesis of SCFAs was associated with epithelial barrier dysfunction, increased mucosal permeability, and activation of inflammatory response.^{83-88,95} The exact mechanism associated with immune activation and increase permeability has not been investigated in detail, but probiotics-treated dysbiotic subjects showed restoration of SCFAs and increase the expression of GPCRs such as GPR41, GPR43, and GPR109A on macrophages. SCFAs and receptors interactions facilitate repair of epithelial barrier function and thereby activating anti-inflammatory signaling cascades.^{81,94,96} Interestingly, Muller et al⁹⁷ demonstrated that luminal microbiota regulate crosstalk between muscularis macrophages (MMs) and enteric neurons to normal regulation of intestinal motility. Related studies also reported severe dysmotility in germ-free rodents,⁹⁸ *Tlr4*^{-/-} and *Myd88*^{-/-} mice,⁹⁹ and our POI model.⁹⁴

Prospective Factors That Could Trigger Activation of Macrophages

Multiple pathways or potential mechanisms activate macrophages to initiate inflammatory phase in POI. First, molecules released in response to cell damage or damage-associated molecular patterns (DAMPs) such as ATP, uric acid, heat-shock proteins, or S100 proteins diffuse in the blood.^{11,100,101} These products through circulation leak out to the site of injury and stimulate muscular monocytes and macrophages.¹⁰⁰⁻¹⁰² Second, cortisol produced through HPA stress axis alter gut microbial diversity (dysbiosis), and impairs gut barrier via modifying TJPs.^{28,31,95} Dysbiosis disturbs the crosstalk between MMs and enteric neurons to activate the network of resident macrophages.^{97,103-107} Third, the activation and degranulation of mast cells in the peritoneal cavity changes its micro-environment. The change in peritoneal environment may directly activate the resident macrophage to release proinflammatory molecules.^{111,108} Fourth, stress induced pCRF release, and its interaction with CRF receptors that could stimulate TLR4 on the macrophages.^{38,109,110} These are some of the major events that would trigger activation of macrophages in surgical intestinal manipulation (IM) (Fig. 3).

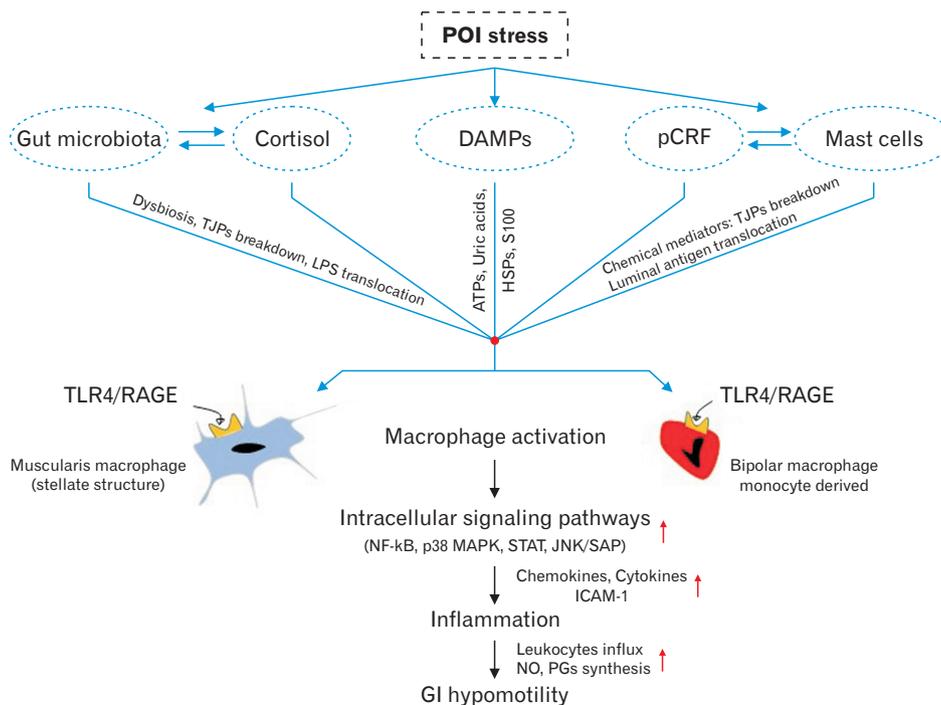


Figure 3. Prospective factors involved in the initiation of inflammatory phase in post-operative ileus (POI). These events or pathways converge to abet vicious cycle of inflammation. DAMPs, damage associated molecular patterns; pCRF, peripheral corticotropin-releasing factor; TJPs, tight junction proteins; LPS, lipopolysaccharide; HSPs, heat shocks proteins; TLR4, Toll-like receptors 4; RAGE, receptor for advanced glycation end products; NF-κB, nuclear factor-kappa B; p38 MAPK, p38 mitogen-activated protein kinase; STAT, signal transducer and activator of transcription; JNK, c-Jun N-terminal kinase; SAP, stress-activated protein; ICAM-1, intercellular adhesion molecules-1; NO, nitric oxide; PGs, prostaglandins; GI, gastrointestinal.

Muscularis Macrophage Activation and Initiation of Intracellular Signaling Pathways

Macrophages play a key role in the pathogenesis of POI. Local inflammation is initiated by macrophages residing in the muscularis layer, which triggers onset of POI (Fig. 4).^{10,11,111-117} These MMs lie in close proximity to neurons within the myenteric plexus, circular and longitudinal smooth muscle layers, and interstitial cells of Cajal.¹¹¹ MMs exist in different subsets based on their morphological (stellate and bipolar) structure, however their precise role is still ambiguous.¹¹¹⁻¹¹⁵ Stellate resident macrophages are long-lived self-maintaining and arise from both embryonic precursors and adult bone marrow-derived monocytes, and persists throughout adulthood.¹¹¹ On the other hand, macrophages lying within the muscle layer exhibit a bipolar morphology.^{111,113} Recently, De Schepper

et al¹¹⁶ demonstrated depletion of self-maintaining macrophages resulted in morphological abnormalities in the submucosal vasculature and loss of enteric neurons, leading to vascular leakage, impaired secretion, and reduced intestinal motility. In the course of surgical stress, the strategic role of self-maintaining MMs had been highlighted and data indicate the central role of MMs in initiating inflammatory cascade.¹¹⁶ The POI model of our study also demonstrated significant increase in the expression of calprotectin in the muscularis externa and serosa in the colon, while expression in the mucosal layer was insignificant (unpublished data). The inflammatory cascade of events in the muscularis externa is initiated by MMs via TLR or receptors for advanced glycation end-products within an hour of IM.^{1,11,111} It activates intracellular signaling pathways such as p38 mitogen-activated protein kinase, c-Jun N-terminal kinases/stress-activated protein, NF-κB, signal transducer and activator of transcription, and extracellular signal-regulated kinase 1/2.^{1,11,118} These pathways are regulated by series of kinases that

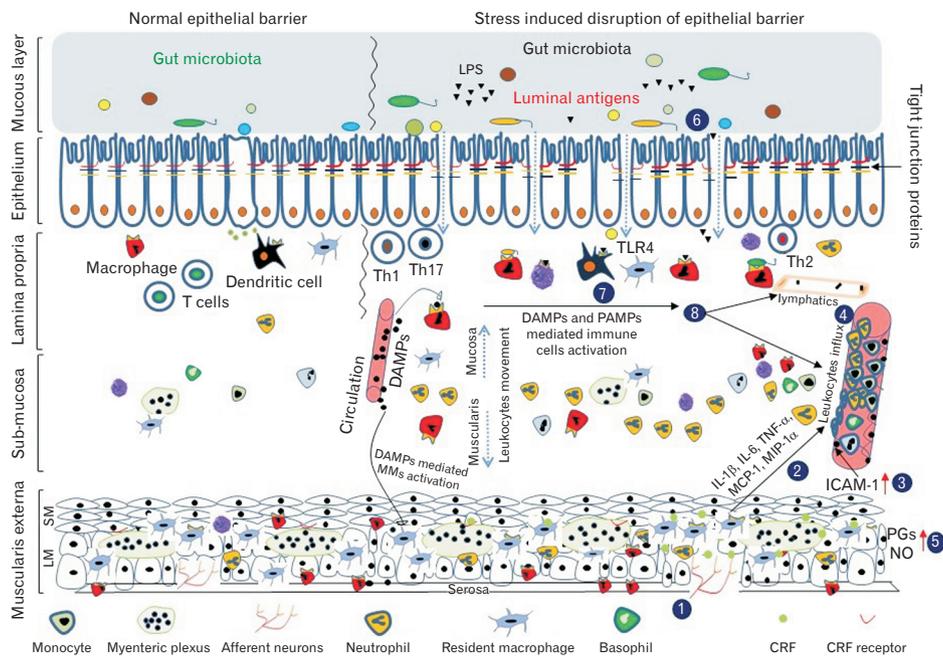


Figure 4. Stepwise events involved in stress induced localized inflammation and barrier dysfunction. ① Stress induced autonomic nerve stimulation, release of peripheral corticotropin-releasing factor (CRF) and muscularis macrophages receptors activation, further strengthened by damage associated molecular patterns (DAMPs) mediated immune cells activation. ② Nuclear activation and release of pro-inflammatory chemokines and cytokines. ③ Upregulation of intercellular adhesion molecules-1 (ICAM-1) in the endothelial cells of blood vessels. ④ Influx of leukocytes in the muscularis. ⑤ Increase synthesis of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzymes in the muscularis resident macrophages, release of inflammatory substances (prostaglandins [PGs] and nitric oxide [NO]) in the muscularis to cause localized inflammation. ⑥ Mast cell and cortisol mediated barrier disruption cause translocation of luminal antigens (lipopolysaccharide [LPS]). ⑦ LPS induce pathogen associated molecular patterns (PAMPs) mediated activation of mucosal immune cells to further exacerbate inflammation. ⑧ The luminal antigen drainage through lymphatics and activation of dendritic cells along activation of inhibitory sympathetic and vagal nerve cause generalized inflammation through gastrointestinal tract to induce post-operative ileus. Th, T helper cells; MM, muscularis macrophages; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; SM, smooth muscle; LM, longitudinal muscle.

finally lead to phosphorylation of transcription factors (TFs). The phosphorylated TFs translocate to the nucleus to start the transcription of proinflammatory genes to express cytokines, chemokines, and other inflammatory molecules.^{1,11,117,119,120} Cytokines TNF- α , IL-6, IL-1 β , and chemokines (monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 α [MIP-1 α]) are secreted by the resident macrophages.^{11,119-121} This inflammatory process favors the upregulation of intercellular adhesion molecules (ICAM-1), on the vascular endothelium lining the muscularis.^{108,118-123} It has been reported that the ICAM-1 mRNA is expressed in the muscularis within 15 minutes of IM,¹²³ may be triggered by mast cells or DAMPs-mediated activation of MMs.¹¹ ICAM-1 along with macrophage-derived chemokines initiate the recruitment of circulating leukocytes such as neutrophils and monocytes to the site of injury in the muscularis externa.^{103,108,119,120-127} Kalff et al¹²¹ also showed the influx of leukocytes into the muscularis started approximately 3 hours after manipulation, gradually increasing until 24 hours postoperatively, with monocytes, neutrophils, and mast cells as predominantly infiltrating leukocytes. The POI model of our study showed association between the degree of inflammation and the recovery time of each segment of GI tract.^{10,128} We detected significant increase in the degree of inflammation in each segment of the GI tract, but the highest degree of inflammation was obtained in the colon.^{10,128} These findings were further substantiated by the predominant expression of calprotectin in the colon 6 hours after

IM in POI groups.^{10,114,128} Calprotectin is generally expressed in neutrophils and macrophages, especially activated macrophages and monocytes in the acute inflammatory state.¹²⁸⁻¹³¹ In addition, Snoek et al⁶⁹ detected the appearance of luminal products in the muscularis externa 6 hours after IM. It validates the observation that translocated bacterial antigens may not trigger muscularis immune responses, but may exacerbate immune responses.^{69,122} The local activated macrophages-mediated molecular inflammatory response is followed by ICAM-1, chemokines-initiated cellular inflammatory phase, leading to the additional recruitment of circulating leukocytes (monocytes and neutrophils) into the muscularis externa.¹²³ Finally, IM induces the synthesis of enzymes such as inducible nitric oxide synthase and cyclooxygenase-2 in the resident macrophages, which mediate the production of nitric oxide, prostaglandins, and arachidonic acid in the intestinal muscular layer.¹³²⁻¹³⁵ The influx of leukocytes along with the accumulation of prostaglandins and nitric oxide inhibits smooth muscle contractility.^{122,133-138} Hence, the role of MMs in triggering inflammatory pathway is further established.

Generalized Inflammation and Inhibition of Contractility in Unmanipulated Segments of Gastrointestinal Tract

However, it remains unclear how local inflammation can lead to a generalized intestinal paralysis. There are few hypotheses that de-

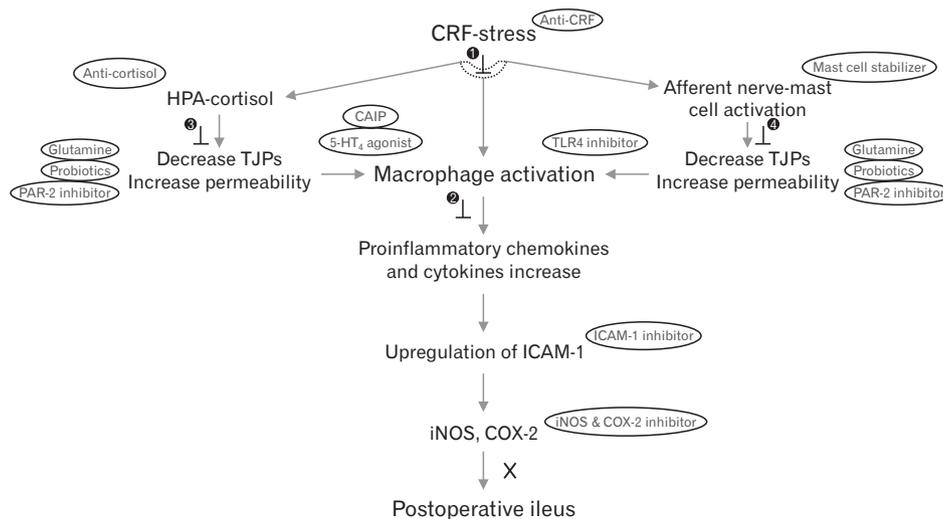


Figure 5. Potential drug target sites in the complex events of post-operative ileus (POI). Several potential sites could be targeted to dampen the effect of POI. Four main intertwined events could be targeted: ① Neuroinflammation, ② Inflammatory pathways, ③ Epithelial barrier disruption, and ④ Gut microbiota. CRF, corticotropin-releasing factor; HPA, hypothalamic–pituitary–adrenal; TJPs, tight junction proteins; PAR-2, protease activated receptor-2; CAIP, cholinergic anti-inflammatory pathway; 5-HT₄, 5-hydroxytryptamine 4 receptor; ICAM-1, intercellular adhesion molecules-1; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2. X means inhibition of POI.

fine this paralysis in unmanipulated area; MMs release IL-12 and activate memory T helper 1 cells to produce IFN- γ , which in turn migrate to intact areas of the gut and spread inflammation.^{132,139,140} Second, lamina propria macrophages transfer the luminal antigens to dendritic cells, which may not lead to mucosal inflammation.¹¹¹ The activated dendritic cells drain into the lymphatics to initiate inflammatory cascade in the unmanipulated area, leading to the pathogenesis of POI in unmanipulated areas.^{1,10-12,69,132,137-140}

Treatment Options and Management of Post-operative Ileus

The review explores and sheds light on neuro-inflammation and related events such as barrier disruption, dysbiosis, and cascading inflammatory reactions. Therefore, these events will be obvious drug targets to manage and treat POI (Fig. 5).

Attenuation of Hypothalamic–Pituitary–Adrenal Axis to Inhibit Corticotropin-releasing Factor Cascading Affects

Attenuation of HPA stress axis may be an important drug target. It would probably inhibit the release of CRF or stimulation of supra-spinal pathway during surgical stress.^{12,28,31} Our study exhibited normalizing effect of prokinetic agent DA-9701 (formulated with *Pharbitis* semen and *Corydalis* tuber) on POI model, showing decreased plasma ACTH level and central CRF expression.¹² We hypothesize that DA-9701 possibly act through central CRF pathway to improve POI.¹² In another stress model, Jung et al³⁰ showed that DA-9701 improves delay in gastric emptying and inhibits plasma ACTH level. Furthermore, Ait-Belgnaoui et al²⁸ exploited a probiotic strain *Lactobacillus farciminis* to attenuate HPA stress axis. The central effects of *L. farciminis* enhance intestinal epithelial barrier and decrease endotoxemia and corticosteronemia.^{28,33} Based on their findings HPA regulatory or anti-cortisol myosin light chain regulatory drugs are key to control enterocytes cytoskeleton contraction-related barrier opening (Fig. 5).

Peripheral Corticotropin-releasing Factor–Corticotropin-releasing Factor Receptors Antagonists

The activation of CRF signaling pathways are known to play a key role in the pathogenesis of POI.^{51,54} Animal studies also established the role of CRF1 in the early phase of postoperative gastric ileus.^{12,54} Peripheral CRF increase epithelial permeability which are mediated via TLR4 and cytokine signaling.^{63,79} These findings

specify a potential new therapeutic venue to alleviate the early phase of postoperative ileus with CRF1 antagonist such as CP-154 526 (Fig. 5).^{12,54}

Mast Cell Stabilization and Regulation of Protease Activated Receptor-2

CRF or neuropeptides-activated mast cells release chemical mediators (tryptase) that activate PAR-2 to initiate inflammatory response and disruption of barrier integrity into the pathogenesis of POI.^{8,10,11,57,67-69} Therefore, mast cell stabilization should be the earliest strategy to be taken into consideration to restore epithelial barrier integrity, decreased permeability, and ameliorate POI recovery.⁶⁹ In our study, the expression of mast cell tryptase increased significantly in the proximal colon, that was effectively decreased by ketotifen (Fig. 5).¹²⁸ Ketotifen is a mast cell-stabilizing agent that blocks the release of mast cell granules.¹⁴¹ Additionally, PAR-2 receptors should be downregulated to modulate pathways involved in gut permeability to prevent POI. Therefore, PAR-2 is a new target in the therapeutic approach of digestive diseases. One such drug is I-287A, a selective and potent PAR-2 inhibitor for the treatment of inflammation.¹⁴²

Restoration of Microbial Diversity

Increasing evidence has indicated that surgical stress causes numerical and compositional shift in the gut microbiota.^{92-94,142} In addition, loss of microbial diversity is involved in surgical complications.¹⁴³ Therefore, it is imperative to compensate the lost microbial population that could repair intestinal barrier damage and promote anti-inflammatory functions. Our study demonstrated that pretreatment of probiotics before surgery restores the beneficial bacterial species, butyrate production, and bowel movement.⁹⁴ The modulation of gut microbiota may help the treatment and prevention of POI (Fig. 5). Separately, water avoidance stress-induced changes in TJPs were restored by the administration of butyrate-producing microbiota.^{39,40} Parada Venegas et al⁹⁵ reported how empirical modulation of the microbiota using prebiotics or probiotics can increase SCFAs-producing bacteria, hence enriching microbial diversity and improving clinical and histological parameters.

Maintenance of Tight Junction Proteins

Gut paracellular permeability is largely determined by alterations of TJPs.^{68,78} So, protecting the TJPs will maintain epithelial permeability and hence enhance POI recovery. In the POI model of our study, there was significant change in the expression of claudin-1 and claudin-2 in both the ileum and proximal colon.^{10,128}

Interestingly, glutamine significantly reversed the level of claudin-1 and claudin-2 expression in both the ileum and proximal colon. Glutamine maintains intestinal tissue integrity, and one of the several mechanisms associated with this function is the induction of the expression of TJPs such as claudin-1, occludin, and zonula occludens.^{76,144} In addition, glutamine also exhibits anti-inflammatory effects by modulating the inflammatory signaling pathways such as NF- κ B, signal transducer, and activator of transcription pathways (Fig. 5).¹⁴⁴

Prevention of Macrophage Activation to Inhibit Inflammation

Multiple pathways or potential mechanisms activate macrophages and other immune cells to initiate inflammatory phase in POI. Practically, multipronged approach should be applied to deplete and inactivate resident macrophages to limit the inflammatory affect.

Prokinetic Mediated Activation of Cholinergic Anti-inflammatory Pathway

One such approach is the activation of cholinergic anti-inflammatory pathway.¹⁴⁵ In our study, lower dose (0.3 mg/kg) of mosapride significantly decreased the leukocytes infiltration as well as calprotectin expression from activated macrophages and neutrophils.¹²⁸ Previous studies indicated anti-inflammatory effect of mosapride that stimulates 5-hydroxytryptamine 4 receptor in myenteric plexus nerve to release acetylcholine (ACh) at the distal end of vagal efferents.^{146,147} ACh in turn inhibits the release of TNF- α , IL-6, MIP-2, and MIP-1 α by macrophages through their α 7 nicotinic ACh receptors to ameliorate inflammation.^{11,115,145-148}

Intracellular Signaling Pathway Inhibitors

Targeting intracellular signaling pathways of the MMs could dampen transcription factors, induction of pro-inflammatory gene expression, and the release of chemokines and cytokines. This may be an interesting alternative approach to treat POI.¹⁴⁹ Semapimod, a p38 mitogen-activated protein kinase inhibitor indeed reduced POI by dampening the expression of the proinflammatory genes MIP-1 α , IL6, MCP-1, and ICAM-1 and decelerate leukocytes influx.¹⁴⁹ In addition, inhibition of phosphorylation of TFs could be the prime drug target to abolish POI effects.¹¹

Downregulation of Intercellular Adhesion Molecules-1

Once the inflammatory cascade is initiated, adhesion molecules such ICAM-1 is upregulated to attract leucocytes from the circula-

tion to impairs smooth muscle contractility. So, a mechanism that prevents ICAM-1 expression, ie, antisense mediated inhibition of ICAM-1, would be an ideal strategy to dampen the effects of POI.¹⁵⁰

Inhibition of Inflammatory Enzymes

The metabolites of inflammatory enzymes have a huge impact on smooth muscle contractility. Therefore, selective inhibitions of enzymes such as inducible nitric oxide synthase or cyclooxygenase-2 could prevent POI.¹³⁶

Conclusions

Taken together, this review highlights the vast influence of neuroinflammation, altered intestinal permeability, and dysbiosis on the inflammatory cascades during surgical stress. The vicious inflammatory cycle helps in the recruitment of leukocytes and increased production of metabolites into the muscularis to impair the smooth muscle contractility and accelerate the pathogenesis of POI. These mechanistic observations may lay the foundation for the discovery of novel and potential therapeutic agents to alleviate the effects of POI. This review concludes that CRF, mast cells, macrophages and gut microbiota should be targeted concurrently for efficient POI management. However, further studies are needed to verify the pathological role of heterogeneous macrophages in the GI tract as well as its interaction with gut microbiota and enteric neurons. In addition, POI-specific changes in the gut bacterial species need to be recognized among microbial population.

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