Abstract. – Protease-activated receptors (PARs) are G-protein-coupled receptors that are activated by the proteolytic cleavage of their N-terminal domain. The new N-terminal sequence that is exposed by proteolysis acts as a tethered ligand, which binds to and activates the receptor. PAR-2 is highly expressed in the gastrointestinal tract, where it is found in endothelial cells, colonic myocytes, enterocytes (both on basolateral and apical membranes), enteric neurons, terminals of mesenteric afferent nerves and immune cells. In the gastrointestinal tract, PAR-2 may be activated by tryptase from mast cells but also by luminal proteases such as trypsin and possibly bacterial proteases.

In addition to effects on motility, ion and mucous secretion, activation of PAR-2 receptors from luminal affects visceral pain. In rats, the intracolonic infusion of PAR-2 agonists (SLIGRL, trypsin) initiates a delayed hypersensitivity to colonic distension. These effects are locally mediated since they are not observed for systemic administration. Interestingly, such pronociceptive effect of local activation of PAR-2 is associated with increased colonic paracellular permeability. Blockade of such increase in permeability, prevents the occurrence of hypersensitivity to rectal distension suggesting that activation of the local immune system by luminal toxins and antigens is responsible for the sensitization of primary afferent terminals to mechanical stimuli. Consequently, blockade of PAR-2 receptors at the periphery and/or inhibition of colonic luminal protease activity may be new interesting targets for the treatment of gut hypersensitivity and IBS.

A recent study has evidenced that stool supernatants from diarrhea predominant IBS patients have a high level of serine-protease activity that increases permeability and colonic hypersensitivity when infused intra-colonically in mice, and these effects are linked to activation of PAR-2 receptors.

These data support a possible role of luminal proteases in the pathogenesis of IBS and give a rationale to target PARs and more specifically PAR-2 as future treatment of IBS.

Key Words: Protease-activated receptors, IBS, Visceral pain, Permeability, Colon, Feces.

Protease-Activated Receptors in the Gut

Protease-activated receptors (PARs) belong to the family of seven transmembrane domain G-protein-coupled receptors that are activated by cleavage of their N-terminal domain by a proteolytic enzyme. The unmasked new N-terminal sequence acts as a tethered ligand that binds and activates the receptor itself. Four types of PARs are described that are selectively cleaved by distinct proteases. Indeed, PAR1, PAR3 and PAR4 are cleaved by thrombin, PAR2 and PAR4 are cleaved by trypsin and tryptase while PAR4 is also cleaved by cathepsin G. PARs are expressed throughout the gastrointestinal (GI) tract on several cell types such as enteroocytes, mast cells, smooth muscle cells, myenteric neurons and endothelial cells. Immunohistochemical studies indicate that PAR2 is localized on both the basolateral and apical side of colonic epithelial cells. PAR2 is also present on nerve terminals of afferent fibres as well as on immunocytes (Figure 1). Moreover, mast cells that are located in close proximity to nerve terminals release proteases and particularly tryptase that cleaves and activates PAR2. It is now established that PAR2 plays an important role in the interplay between nerves, immunocytes, mast cells and epithelial cells within the gut wall (Figure 1).
Effects on the Digestive Tract

Experimentally, activation of PAR2 is associated with a variety of effects on the major digestive functions known to be altered in human digestive pathologies.

Secretion
Activation of PAR2 on the luminal side of enterocytes triggers intestinal water secretion through a prostaglandin E2-dependent mechanism in rats or by a direct cellular mechanism in humans. In addition, luminal activation of PAR2 stimulates mucus secretion by a nerve-mediated mechanism.

Motility
Activation of PAR2 has been found to modulate GI motility in different ways (see review by Kawabata et al.). For example, activation of PAR1 and PAR2 contracts rat and mouse gastric smooth muscle but reduces contractility of both circular and longitudinal colonic smooth muscle. It accelerates GI transit, at least in mice.

Inflammation
At the gastric level, PAR2 activation plays a protective role by stimulating mucus secretion through a mechanism involving capsaicin-sensitive neurons, increases gastric mucosal blood flow, and inhibits gastric acid secretion evoked by carbachol or pentagastrin. Intracolonic activation of PAR2 leads to colonic inflammation in mice, depending upon the time elapsed from the application suggesting an indirect inflammatory mechanism involving both capsaicin-sensitive sensory neurons, tachykinins and nitric oxide. Bacterial translocation to peritoneal organs is also one of the features observed after PAR2 activation on the luminal side of the colon, suggesting an alteration in mucosal permeability that may trigger or participate in long-term colonic inflammatory reactions.

Mechanical Hypersensitivity
PAR2 activation on the luminal side was shown to produce a delayed increase in colonic sensitivity to distension in rats. This hypersensitivity to distension was dose-related and lasted more than 24 hours. In addition, it was found that systemic administration of PAR2 agonists was unable to reproduce the effect of luminal PAR2 agonism and that the PAR2-evoked hypersensitivity was associated with an increase in gut paracellular permeability.

Mucosal Permeability
Both the transcellular and paracellular permeability of the GI mucosa is relevant not only to
nutritional aspects but also to the maintenance of immune balance between the internal and external milieu. While the transcellular pathway often requires an active transport and is therefore elective, the paracellular pathway acts as a filter and is the major way of non-specific macromolecule transport. Many human digestive pathologies are associated with alterations of gut paracellular permeability including celiac disease, Crohn’s disease, ulcerative colitis and irritable bowel syndrome. In all these digestive pathologies, increased permeability is found to be associated with activation of the mucosal immune system. Abnormal gut paracellular permeability is considered as a primary event in the genesis of diseases such as necrotizing enterocolitis and to some extent celiac disease. In other diseases such as Crohn’s disease, there is no direct proof that paracellular permeability plays a pivotal role. However, increased permeability is often present in familial relatives not developing the disease and was found associated with NOD2 variants in both familial and sporadic Crohn’s disease, which suggests that altered permeability is both a genetic and a non-genetic factor favoring the occurrence of the disease. Furthermore, it is currently considered that altered permeability may greatly contribute to the occurrence of relapses. Increased permeability precedes symptoms with a correlation between clinical disease activity index and intestinal/colonic tight junction leakage.

Importance of Permeability in IBS

Recently, a role of altered permeability in the genesis of IBS symptoms has been conceptualized. Indeed, during the last 4-5 years, altered intestinal and/or colonic permeability has been identified in all IBS patients according to Rome I criteria or in subsets of either diarrhea-predominant (IBS-D) or post-infectious IBS patients. The relationship with IBS symptoms has not yet been established in humans. One of the reasons for this lack of information is linked to the methodology used to determine mucosal permeability, i.e. the use of different markers such as sugars or ¹⁸⁷⁶⁶ena for specific detection of the site or type of paracellular permeability alterations. However, animal studies have demonstrated that factors which enhance colonic permeability such as stress, lipopolysaccharide, bile salts or the activation of PARs initiate a long-term hypersensitivity of the gut to distension and, conversely, pharmacological blockade of increased permeability prevents hypersensitivity to colorectal distension. Animal studies also confirm that an increase of tight junction permeability is associated with mucosal micro-inflammation and bacterial translocation.

These data provide a rationale to investigate the possible endogenous and luminal factors that may be responsible for increased tight junction permeability.

Factors Controlling Gut Permeability

Gut paracellular permeability is influenced by both endogenous and luminal factors. Mucosal mast cells release mediators able to increase gut macromolecular passage and intercellular permeability in stressed rats, and these stress-induced permeability changes involve local activation of corticotropin-releasing factor (CRF) receptors. Neonatally stressed rats also present long-term alterations of colonic permeability under the dual control of CRF and nerve growth factor (NGF). In mice, the stress-induced increase in paracellular permeability depends upon the presence of activated T-cells in the lamina propria and the subsequent release of interferon-γ (IFNγ) which is responsible for the final contraction of the epithelial cell cytoskeleton through activation of myosin light chain (MLC) kinase that phosphorylates myosin light chain. Based on the use of oral antibiotics, the commensal microflora has also been shown to play an important role in the control of basal and stress-increased permeability.

PAR2 Activation-Induced Changes in Permeability

In the healthy gut in vivo, PAR2 is present on both the apical and basolateral membrane of the enterocytes. Many experiments have been performed in vitro, and it is not clear whether in vivo one way of activation (apical vs. basolateral) predominates over the other. Moreover, the expression of PAR2 on cultured tumoral cell lines may be different from in vivo.

Supernatants from degranulated mast cells increase intracellular Ca in colonocytes, an in-
crease which was blocked by a trypsin inhibitor or prevented by previous activation of PAR2 by a PAR2 agonist. When applied to the basolateral surface of colonocytes, PAR2 agonists and mast cell supernatants decreased transepithelial resistance, increased transepithelial flux of macromolecules, and induced redistribution of tight junction ZO-1, occludins and perijunctional F-actin. When mast cells were co-cultured with colonocytes, mast cell degranulation increases paracellular permeability of colonocytes, an effect prevented by a trypsin inhibitor. Extracellular signal-regulated kinases (ERK1/2) and beta-arrestins, which recruit ERK1/2 to PAR2 in endosomes and retain ERK1/2 in the cytosol, have been implicated in PAR2-mediated alterations of permeability. For instance, an ERK1/2 inhibitor abolishes the effects of a PAR2 agonist on permeability and redistribution of F-actin. In addition, down-regulation of beta-arrestins with small interfering RNA inhibits PAR2-induced activation of ERK1/2 and suppresses PAR2-induced changes in permeability. Thus, mast cells signal to colonocytes in a paracrine manner by release of tryptase and activation of PAR2. PAR2 couples to beta-arrestin-dependent activation of ERK1/2, which regulates reorganization of perijunctional F-actin to increase epithelial permeability. These mechanisms may explain the increased epithelial permeability of the intestine during stress and inflammation. In vivo, the apical expression of PAR2 has been shown to fluctuate during chronic oral treatment (12 days) with a mixture of non-absorbable antibiotics.

In 2002, Coelho et al. have shown for the first time in rats that intracolonic infusion of the PAR2-activating peptide SLIGRL triggers a dose-related increase in colonic paracellular permeability and that this effect cannot be reproduced by similar doses injected intraperitoneally. In mice, a dose of 5 µg of SLIGRL infused intracolonically increases colonic permeability while a 10 times higher dose is required to induce an inflammatory reaction of the mucosa. Moreover, while afferent nerve integrity and INFγ deficient mice that the inflammatory reaction depends upon the presence of T-lymphocytes and INFγ. Moreover, the pro-inflammatory action of PAR2 activation depends upon the pathophysiological state of the mucosa. It has been confirmed in rats that SLIGRL infused into the duodenum causes mucosal inflammation, whereas it suppresses the inflammatory damage induced by ischemia/reperfusion.

**Luminal Proteases, PAR2 and IBS**

Proteases are present in great amounts in the GI tract. In addition to their digestive role in protein degradation, they play a role as signaling molecules regulating cell functions by cleaving PARs. PARs are activated by a variety of proteases, such as digestive enzymes (trypsin and trypsinogen), proteases released from mast cells and neutrophils, and proteases of the coagulation cascade. Bacteria may degrade these enzymes but are also able to release serine-proteases into the lumen. The impact of the microflora on the colonic luminal level of proteases was shown by the effect of antibiotic treatment of rats. Thus, the luminal serine-protease activity was attenuated after 2 weeks of oral treatment with a mixture of ampicillin and neomycin, and this change was
associated with a reduction of basal colonic permeability and a down-regulation of PAR2. Such a decrease in permeability was also observed after intracolonic infusion of a cocktail of serine-protease inhibitors.

All these data lead us to hypothesize that bacterial proteases, or even luminal proteases of endogenous origin, may act on PARs present on the apical side of colonocytes. In animals, oral antibiotic treatment is associated with a down-regulation of PAR2 expressed by epithelial cells and with reduced basal permeability and reduced responses to luminal activating factors. Over-expression of PAR2 was observed in biopsies from patients with inflammatory bowel disease (IBD), suggesting a pathophysiological role of PAR2 in the development of colonic inflammation. A recent study has highlighted the high level of serine-proteases found in supernatants from feces of both IBS and IBD patients. A subgroup analysis revealed that this increase was selective for IBS-D patients and not observed in constipated and alternating IBS patients. The increase in serine-proteases which is similar to that observed in patients with ulcerative colitis is not observed in the feces of patients suffering from infectious diarrhea. In contrast to the fecal supernatants from ulcerative colitis patients, those of IBS-D patients are not associated with any significant elevation of inflammatory markers such as myeloperoxidase and calprotectin. The origin of this increase of fecal serine-protease activity has not been clearly established. The pancreatic elastase-1 concentration has been found to be similar in feces from IBS-D patients and controls. Likewise, mast cell tryptase activity and secretory leukocyte protease inhibitor concentration were normal. Consequently, the source of fecal serine-protease activity which is increased in IBS-D does not seem to be from pancreas, mast cells or immune cells. The hypothesis of a bacterial origin of increased protease activity is indeed possible, since it is well established that colonic commensal bacteria release considerable amounts of proteases that are partly degraded by host proteases and bacterial peptide hydrolases.

The pathophysiological significance of the increased colonic serine-protease activity found in IBS-D patients has been investigated in mice both in vitro and in vivo. Interestingly, diluted fecal supernatants from IBS-D patients trigger an increase in paracellular permeability when ap-

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Figure 2. Implications of PAR2-induced alteration of mucosal permeability in local immune function and visceral pain. PAR2-induced phosphorylation of MLC is a short lasting phenomenon (15 to 120 min) but restoration of permeability linked to resynthesis and migration of new junction proteins may last more than 12 hours during which there is an activation of the resident immune system and attraction of new immunocytes. This micro-inflammation releases mediators from T-cells (IFN-γ) and mast cells (tryptase) that may prolong MLC kinase activation. The micro-inflammation sensitizes nerve terminals that may in turn favor neurogenic inflammation and maintain a long-term hypersensitivity to mechanical stimuli.
plied to the mucosal side of mice colonic mucosa in Ussing chambers. This increase is suppressed after previous incubation with a cocktail of serine-protease inhibitors and is not observed in PAR2-deficient mice. IBS-D supernatants infused intracolonically in mice also produce both a delayed colonic hypersensitivity to distension and a change in the expression of colonic tight junction proteins with activation of MLC phosphorylation. Although until now no correlation has been established between the fecal level of serine-protease activity and the intensity/frequency of symptoms, it is tempting to speculate, based on the animal data, that the fecal serine-protease level contributes to the genesis of IBS symptoms and particularly to abdominal pain. Interestingly, a decreased colonic potential difference, corresponding to an increase in permeability, has been found in IBS-D and ulcerative colitis patients compared with healthy subjects. We can speculate that this decreased potential difference is linked to elevated protease activity and PAR2 activation.

Cenac et al. have recently shown that the colonic mucosa of IBS patients contains high levels of proteases with a high expression of trypsin-like activity resulting from stress-induced stimulation of exocrine pancreatic secretion and (ii) particularly trypsin which is a selective activator of PAR2 that may reach the colonic lumen. All these data suggest that the primary mucosal epithelial response to PAR2 activation depends upon luminal stimuli and apical membrane receptor activation, which triggers mucosal immune stimulation, involves mast cells and tryptase, and is able to maintain a high degree of gut “porosity”.

Conclusions

Activation of PAR2 plays an important role in digestive pathology. In addition to its role in nerve-immunocyte communication in the gut wall, activation of epithelial cell PAR2 may be important in the pathogenesis of organic and functional GI disorders particularly by affecting paracellular permeability. Increased intestinal and colonic permeability is associated with the passage of luminal antigens, toxins and bacteria that activate the mucosal immune system with immunocyte recruitment and release of inflammatory mediators. The fact that luminal proteases found at elevated level in colonic contents from IBS and ulcerative colitis patients are able to activate PAR2 to promote increased permeability and gut sensitivity suggests that PAR2 is a relevant new target in the therapeutic approach to digestive diseases.

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Protease activated receptor 2: a new target for IBS treatment


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