Basic and Translational Research on Proteinase-Activated Receptors: Implication of Proteinase/Proteinase-Activated Receptor in Gastrointestinal Inflammation

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Abstract. Recently, the role of serine proteinases in the pathogenesis of inflammation and autoimmune diseases via interaction with the proteinase-activated receptor (PAR) has attracted attention. Activation of PAR has a pro-inflammatory effect through the overproduction of inflammatory cytokines such as interleukin (IL)-6 and IL-8. PAR² activation in human esophageal epithelial cells by trypsin induces NFκB– and AP-1–dependent IL-8 production in association with activation of p38 MAPK and ERK1/2, suggesting that esophageal inflammation may be induced by PAR² activation via reflux of trypsin. It has been also proposed that Helicobacter pylori (H. pylori) induces PAR expression in the gastric epithelial cells and H. pylori–derived serine proteinase promotes IL-8 production via PAR in the epithelial cells. In addition, an increase of PAR-dependent IL-8 production has been observed in H. pylori–infected human gastric mucosa, suggesting an important role for PAR² in the modulation of gastric inflammation associated with H. pylori. Recent studies have strongly indicated that tryptase and PAR are implicated in the pathogenesis of inflammatory bowel disease and experimental colitis. We demonstrated that anti-tryptase therapy may become a new therapeutic strategy in human ulcerative colitis. Thus, the role of PAR in the gastrointestinal tract has been gradually clarified, but further investigations are needed because the receptor has a variety of functions.

Keywords: proteinase-activated receptor (PAR), esophagitis, Helicobacter pylori, colitis, proteinase inhibitor

Introduction

It has recently been clarified that various proteinases are not only involved in digestion of the extracellular matrix but also in the process of intracellular signaling via proteinase-activated receptor (PAR), thus having a variety of roles in inflammatory/immune responses (1, 2). This receptor is expressed in various organs, including the gastrointestinal tract, and its activation results in proinflammatory effects, including cytokine production (3, 4), vasodilatation (5), edema (6), and leukocyte-endothelial interactions (6, 7).

In this review, we summarize the implications of the proteinase/PAR pathway, especially PAR², in gastrointestinal inflammation.

PAR activation and its function

PAR mediates the cellular actions of serine proteinases, and it is a seven transmembrane trimer G-protein–coupled receptor that is activated by specific proteinases. To date, 4 members of the PAR family (PAR¹, PAR³, PAR⁴, and PAR⁵) have already been cloned. While PAR¹, PAR³, and PAR⁴ are mainly activated by thrombin, PAR² is activated by trypsin, mast cell tryptase, and coagulation factors (VIIa, Xa). Platelets, vascular endo-
thelial cells, and vascular smooth muscle cells are known to express PAR. Recently, it has been reported that PAR is also expressed by epithelial cells in the bronchi, uterus, urinary bladder, and gastrointestinal tract, where it is involved in various pathological responses (2, 8). When activation occurs, the extracellular N-terminal peptide chain of PAR is cut at a specific site by the agonist proteinase and the exposed N-terminal specifically combines with the extracellular second loop of the receptor itself (tethered ligand) to initiate intracellular signaling (Fig. 1). Although there are various intracellular signal transduction pathways after PAR activation, these usually involve G-protein-mediated activation of phospholipase C, an increase of the cytoplasmic calcium concentration, and activation of protein kinase C (8, 9).

It has been shown that PAR (particularly PAR1 and PAR2) is widely distributed in the gastrointestinal tract and is involved in regulating salivary gland secretion, mucus and pepsin production, gastrointestinal motility, pancreatic juice excretion, and small intestinal ion transport (10). In addition, the activation of PAR results in proinflammatory reactions via production of cytokines such as interleukin (IL)-6 and IL-8 and prostaglandin production (3, 4).

**Trypsin/PAR pathway in reflux esophagitis**

In recent years, the pathology of reflux esophagitis has been studied in terms of inflammation, and increased expression of IL-8, a potent activating factor of neutrophils, has been reported in esophageal biopsy specimens from patients with reflux esophagitis (11–15). Reflux of proteinases such as trypsin, as well as gastric acid and bile acids, has been reported to cause damage to the esophageal mucosa, but the mechanism of this injurious action has not yet been elucidated completely.

We have determined PAR2 expression in normal human esophageal epithelial cells (HEEC) and evaluated the mechanism of IL-8 production by trypsin-PAR2 interaction in HEEC (16). IL-8 was produced in a dose-dependent fashion when cells were stimulated with a PAR2 agonist such as trypsin or SLIGKV-amide. Blocking antibody to PAR2, camostat mesilate (a trypsin inhibitor), p-38 mitogen-activated protein kinase (MAPK) inhibitors, or ERK1/2 inhibitors reduced IL-8 production from trypsin-stimulated HEEC. Mutation of the NFκB–, AP-1–, and NF-IL6–binding site on the IL-8 gene promoter abrogated the induction of luciferase activities stimulated with trypsin by 100%, 80%, and 50%, respectively. These results indicate that PAR2 activation in HEEC by trypsin induces NFκB– and AP-1–dependent IL-8 production in association with activation of p38 MAPK and ERK1/2, suggesting that esophageal inflammation may be induced by PAR2 activation via reflux of trypsin, as well as by bile acids (16, 17) (Fig. 2).

**H. pylori–derived proteinase/PAR in IL-8 production from gastric epithelial cells**

*H. pylori* plays an important role in the pathogenesis of chronic gastritis, peptic ulcer, gastric adenocarcinoma, and mucosa-associated tissue lymphoma. The attachment of *H. pylori* to gastric epithelial cells leads to the production of proinflammatory chemokines such as...
IL-8, which in turn cause the activation and recruitment of neutrophils to the site of infection (18, 19). Several studies indicate that the attachment of *H. pylori* to gastric epithelial cells induces IL-8 production through its virulence factors such as CagA, lipopolysaccharide (LPS), OipA, and heat-shock protein (HSP)-60 derived from *H. pylori* (20 – 25).

Recently, we demonstrated the increased PAR expression and the PAR-mediated IL-8 production in *H. pylori*–infected gastric epithelial cells (26). In our study using cultured gastric epithelial cells, PAR$_2$ mRNA and protein were constitutively expressed in unstimulated MKN45 cells. The treatment of cells with *H. pylori* resulted in a significant increase in PAR$_2$ expression. In addition, trypsin (a natural PAR$_2$ agonist), SLIGKV-amide (a synthetic PAR$_2$ agonist), *H. pylori* live bacteria, or *H. pylori* culture supernatant significantly induced IL-8 production from MKN45 cells. *H. pylori*–induced IL-8 production was inhibited by nafamostat mesilate (a serine proteinase inhibitor), neutralizing antibody to PAR$_2$, and in PAR$_2$-deficient cells treated with siRNA. These results reveal that *H. pylori*–derived proteinases activate gastric epithelial cells to produce inflammatory cytokines through activated PAR$_2$.

**PAR expression and IL-8 production in *H. pylori*–infected gastric mucosa**

We have also investigated PAR expression and IL-8 production after *H. pylori* infection using human gastric mucosal biopsy specimens (30). Both PAR$_1$ and PAR$_2$ were expressed by the noninfected gastric mucosa, but the expression of these receptors was markedly upregulated with an increase of IL-8 production in *H. pylori*–infected gastric mucosa (Table 1). A positive correlation was observed between the level of PAR$_1$ or PAR$_2$ expression and the mucosal IL-8 content. Furthermore, the expression of PAR$_1$/PAR$_2$ and IL-8 was decreased by eradication of *H. pylori*. These results indicate that *H. pylori* infection induces gastric mucosal PAR expression and accelerates IL-8 production, suggesting the involvement of PAR in the pathogenesis of gastritis associated with *H. pylori*.

Kawabata et al. demonstrated that PAR$_1$ expressed in the gastric mucosa has an inhibitory effect on gastric acid secretion while increasing gastric mucosal blood flow (10). Toyoda et al. have reported that PAR$_1$ protects the gastric mucosa because the secretion of gastric mucin and PGE$_2$ is promoted by administration of a PAR$_1$-activating peptide (31). In addition, Kawabata
et al. have reported that PAR$_2$ has a role in both aggressive and protective responses for the gastric mucosa (32, 33). Further studies are needed to determine the role of PAR in human gastric mucosal disturbance.

**Anti-proteinase/PAR$_2$ therapy in colonic mucosal inflammation**

Tryptase is one of the serine proteinases that exhibit trypsin-like activity. It is stored almost exclusively in the secretory granules of mast cells (34). Tryptase acts to induce microvascular leakage (35), the chemotaxis of inflammatory cells (36), and stimulates the release of IL-6 and -8 through the MAPK/AP-1 pathway (37). It has been reported that tryptase secretion is significantly increased in the colonic tissue of ulcerative colitis (UC) patients (38) and that the intracolonic administration of PAR$_2$ agonists such as tryptase and trypsin induced an inflammatory reaction characterized by granulocyte infiltration, increased wall thickness, tissue damage, and elevated T-helper cell type 1 cytokine (39, 40). These results strongly indicate that tryptase is involved in the pathogenesis of inflammatory bowel disease (IBD), suggesting that the proteinase/PAR$_2$ pathway may be a therapeutic target in IBD.

The effect of anti-trypase therapy on experimental colitis and human IBD has been demonstrated. The result of a pilot study has revealed that systemic administration of APC2059, a specific tryptase inhibitor, is safe and there is evidence of activity in the treatment of UC (41).

Recently, Mori et al. (42) and Sendo et al. (43) have reported that the reduced dosing of nafamostat mesilate (NM), a proteinase inhibitor widely used for the treatment of acute pancreatitis and disseminated intravascular coagulation, could lead to the selective inhibition of tryptase, with a $K_i$ value of 95.3 pM (9.6 $\times$ 10$^{-11}$ M). Using the reduced dosage of NM, we determined the therapeutic effect of tryptase inhibitor in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (44, 45). In this experiment, all rats were divided into three groups (sham, TNBS, and NM-treated group) after the induction of colitis. Tryptase-positive mast cells were remarkably accumulated in the colonic mucosa after TNBS administration. One milliliter of NM solution (10$^{-11}$ M) was administered anally once a day for 6 days beginning at 24 h after TNBS instillation. Colonic mucosal inflammation determined by damage score, body weight, wet weight, myeloperoxidase (MPO) activity (an index of neutrophil accumulation), and TNF-$\alpha$ was assessed 7 days after TNBS administration. All three NM groups were significantly improved by the intrarectal administration of a low dose of NM. These inhibitory effects of NM

### Table 1. PAR mRNA and IL-8 mRNA levels in the human gastric mucosa infected with *H. pylori* compared to those in non-infected gastric mucosa

<table>
<thead>
<tr>
<th>Group</th>
<th>$H. pylori$ negative</th>
<th>$H. pylori$ positive</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antrum</td>
<td>1.51 $\times$ 10$^{-3}$ ± 0.20</td>
<td>2.66 $\times$ 10$^{-3}$ ± 0.39</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td>Body</td>
<td>1.38 $\times$ 10$^{-3}$ ± 0.17</td>
<td>3.41 $\times$ 10$^{-3}$ ± 0.64</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td>Antrum</td>
<td>2.06 $\times$ 10$^{-2}$ ± 0.31</td>
<td>2.71 $\times$ 10$^{-2}$ ± 0.43</td>
<td>$P=0.27$</td>
</tr>
<tr>
<td>Body</td>
<td>0.81 $\times$ 10$^{-2}$ ± 0.15</td>
<td>1.53 $\times$ 10$^{-2}$ ± 0.25</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td>Antrum</td>
<td>0.19 $\times$ 10$^{-2}$ ± 0.02</td>
<td>1.10 $\times$ 10$^{-2}$ ± 0.39</td>
<td>$P&lt;0.05$</td>
</tr>
<tr>
<td>Body</td>
<td>0.15 $\times$ 10$^{-2}$ ± 0.02</td>
<td>1.92 $\times$ 10$^{-2}$ ± 1.16</td>
<td>$P&lt;0.05$</td>
</tr>
</tbody>
</table>

### Table 2. Effects of NM enema on 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Damage score (0–8)</th>
<th>Body weight increase (g/week)</th>
<th>Wet weight (mg/8 cm)</th>
<th>MPO activity (mU/mg prt.)</th>
<th>TNF-$\alpha$ (pg/mg prt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0 (0)</td>
<td>47.0 ± 2.55</td>
<td>480 ± 20.0</td>
<td>1.74 ± 0.45</td>
<td>1.39 ± 0.08</td>
</tr>
<tr>
<td>TNBS</td>
<td>7.5 (5–8)*</td>
<td>8.12 ± 5.82*</td>
<td>1125 ± 78.5*</td>
<td>4.90 ± 0.85*</td>
<td>20.75 ± 5.35*</td>
</tr>
<tr>
<td>TNBS + NM</td>
<td>3 (2–4)*</td>
<td>30.0 ± 1.97*</td>
<td>570 ± 30.0*</td>
<td>2.29 ± 0.26*</td>
<td>4.44 ± 1.34*</td>
</tr>
</tbody>
</table>

Rats in the sham group received an enema of physiological saline instead of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Rats in the NM group received an intra-colonic injection of NM solution (10$^{-11}$ mol/L) daily for 6 days. Rats in the TNBS and sham groups received an intra-colonic injection of vehicle (distilled water) instead of NM. Colonic inflammation was assessed 1 week after TNBS administration. The damage score for each rat was assigned according to the Moriss (46) and Yoshida (47) criteria, and data were expressed as the median (range). The other data were expressed as the mean ± S.E.M for 10 rats in each group. *$P<0.01$, when compared with the sham group receiving the vehicle; *$P<0.01$, when compared with the TNBS group receiving the vehicle. Reproduced from Ref. 45 with permission.
on TNB-induced colitis were almost equal to those of a 5-aminosalicylic acid (5-ASA) enema, which is a standard therapy in human IBD.

In addition, NM enema improved clinical and endoscopic findings in UC patients that were resistant to conventional therapy such as corticosteroids and sulfasalazine/5-ASA (45). In the case of a 70-year-old patient with UC, follow-up colonoscopy performed 14 days after beginning the NM enema revealed a reduction of erosions and edema in the sigmoid colon and rectum (Fig. 4). UC disease activity index (UCDAI) (48) decreased from 9/12 to 2/12 (Fig. 5). In the immunohistochemical study, the number of tryptase-positive cells decreased in the colonic mucosa compared with the numbers present before NM enema therapy (Fig. 4). These evidences in both experimental colitis and human UC suggest that anti-tryptase therapy may have excellent potential as a new therapeutic strategy.
for the treatment of IBD resistant to conventional therapy such as corticosteroids and sulphasalazine/5-ASA. Corticosteroids and sulphasalazine/5-ASA have been proposed to be effective for the cure of IBD via non-specific anti-inflammatory effects. Low dose of NM selectively inhibiting tryptase activity has a therapeutic specific anti-inflammatory effects. Low dose of NM proposed to be effective for the cure of IBD via non-

References

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