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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Acinar Cell Production of Leukotriene B_4 Contributes to Development of Neurogenic Pancreatitis in Mice

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SUMMARY

The initiation of neurogenic inflammation in pancreatitis is unknown. This work shows that pancreatic acinar cells express 5-lipoxygenase and produce leukotriene B_4 (LTB_4). 5-lipoxygenase inhibition reduces LTB_4 secretion and pancreatitis, indicating that LTB_4 mediates neurogenic pancreatic inflammation.

BACKGROUND & AIMS: In the pancreas, activation of primary sensory nerves through the transient receptor potential vanilloid-1 (TRPV1) ion channel contributes to the early stages of development of pancreatitis. Little is known about the mechanism by which this occurs. We investigated whether leukotriene B_4 (LTB_4) is an endogenous agonist of TRPV1 and mediates pancreatitis.

METHODS: Acute inflammation was induced in the pancreata of *Trpv1*$^{-/-}$ mice and their wild-type littermates by retrograde infusion of the main pancreatic duct with 2% sodium taurocholate (NaT) or intraperitoneal injections of caerulein. Mice were also given injections of resiniferatoxin (an excitotoxin that desensitizes TRPV1) or MK886 (a drug that inhibits LTB_4 biosynthesis). Pancreatic tissues and plasma were collected and analyzed.

RESULTS: Retrograde perfusion of the main pancreatic ducts of wild-type mice with NaT caused severe acute pancreatitis; the severity was reduced by coadministration of resiniferatoxin. *Trpv1*$^{-/-}$ mice developed a less severe pancreatitis after NaT administration compared with controls. Administration of MK886 before perfusion with NaT also significantly reduced the severity of pancreatitis in wild-type mice. Pancreatic tissues from mice given NaT had a marked increase in the level of 5-lipoxygenase immunoreactivity specifically in acinar cells. Bile acid and caerulein induced secretion of LTB_4 by cultured pancreatic acinar cells; MK886 inhibited this process.

CONCLUSIONS: Administration of caerulein or intraductal bile acids in mice causes production of LTB_4 by pancreatic acinar cells. This activates TRPV1 on primary sensory nerves to induce acute pancreatitis. *(Cell Mol Gastroenterol Hepatol 2015;1:75–86; http://dx.doi.org/10.1016/j.jcmgh.2014.11.002)*

Keywords: Gallstone Pancreatitis; Mouse Model; Neurogenic Inflammation; Secretagogue; Vanilloid.

Neurogenic inflammation is caused by the local release of inflammatory neuropeptides such as substance P and calcitonin gene-related peptide from afferent sensory neurons. These peptides produce vasodilation and edema and lead to neutrophil recruitment in the affected tissue. In the pancreas, we have shown that activation of the transient receptor potential vanilloid-1 (TRPV1) ion channel expressed by primary sensory nerves plays a role in the inflammatory cascade in pancreatitis.[1–5] We have proposed that the respective inflammatory insults cause the release of an endogenous TRPV1 agonist in the pancreas resulting in TRPV1 activation in pancreatic primary sensory nerves, which in turn release proinflammatory neurotransmitters such as substance P[6,7] both peripherally within the pancreas itself to cause inflammation and centrally within the spinal cord to cause pain.[8,9] However, the endogenous ligand that activates TRPV1 in pancreatitis is unknown. After the cloning and molecular characterization of TRPV1, it was discovered that leukotriene B_4 (LTB_4)[10] can activate this receptor, raising the possibility that LTB_4 could be the endogenous ligand for TRPV1 after pancreatic injury. Despite this possible role, there is very limited evidence that LTB_4 is found in the pancreas.

The mechanisms involved in the pathophysiology of acute pancreatitis in humans are poorly understood. There are multiple animal models of experimental acute pancreatitis, but the relevance of most of them to human pancreatitis is unclear. One apparent exception is retrograde infusion of bile acids into the main pancreatic duct in rats and mice.[1–3] It has been proposed that this experimental model is closely related to human biliary pancreatitis, which is thought to be caused by bile reflux into the pancreatic duct secondary to gallstone disease.[4] There is evidence that acute pancreatitis

Abbreviations used in this paper: 5-LO, 5-lipoxygenase; FLAP, 5-lipoxygenase-activating protein; Gpbar-1, G protein-coupled bile acid receptor-1; LTB_4, leukotriene B_4; MK886, 1[(4-chlorophenyl) methyl]-3-[(1,1-dimethylethyl)thio]-α,α-dimethyl-5-(1-methylethyl)-1*H*-indole-2-propanoic acid; MPO, myeloperoxidase; NaT, sodium taurocholate; PCR, polymerase chain reaction; PPARα, peroxisome proliferator-activated receptor α; rER, rough endoplasmic reticulum; RTX, resiniferatoxin; TBS, Tris-buffered saline; TLCS, taurolithocholic acid 3-sulfate disodium salt; TRPV1, transient receptor potential vanilloid-1.

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2014.11.002

induced by bile acid in the mouse is initiated by the binding of bile acids to a G protein-coupled receptor in the pancreatic acinar cell apical plasma membrane. In this model, the bile acid receptor G protein-coupled bile acid receptor-1 (Gpbar-1, also known as TGR5) when activated by binding bile acids generates pathologic intracellular calcium transients, resulting in intra-acinar cell zymogen activation and acinar cell injury.[5]

In the present study, we tested the hypothesis that the mechanisms involved in acute pancreatitis induced by bile acid or secretagogue hyperstimulation include generation of an endogenous TRPV1 agonist that activates primary sensory nerves to injure the pancreas. We found that bile acids and caerulein hyperstimulation cause LTB_4 synthesis and secretion from pancreatic acinar cells and that this endogenous release of LTB_4 activates TRPV1 to contribute to acute pancreatitis.

Materials and Methods

These studies were approved by the Duke University Institutional Animal Care and Use Committee.

Animals

Mice in which the *Trpv1* gene has been deleted (*Trpv1*$^{-/-}$) were a kind gift of Dr. D. Julius, University of California–San Francisco. *Trpv1*$^{-/-}$ mice and wild-type littermates were backcrossed onto a C57BL/6 background and genotyped as described previously elsewhere.[11]

Materials

Sodium taurocholate (NaT), taurolithocholic acid 3-sulfate disodium salt (TLCS), and resiniferatoxin (RTX) were purchased from Sigma-Aldrich (St. Louis, MO), MK886 (1[(4-chlorophenyl)methyl]-3-[(1,1-dimethylethyl)thio]-α,α-dimethyl-5-(1-methylethyl)-1*H*-indole-2-propanoic acid) was purchased from Tocris Bioscience (Ellisville, MO), and leukotriene B_4 (LTB_4) enzyme immunoassay kits were purchased from Cayman Chemical (Ann Arbor, MI). Purified mouse monoclonal anti-human 5-lipoxygenase (5-LO) primary antibody, biotinylated goat anti-mouse Ig secondary antibody, and streptavidin-horseradish peroxidase were purchased from BD Biosciences (San Jose, CA).

Induction of Acute Pancreatitis

Acute inflammation of the pancreas was stimulated by retrograde infusion of the main pancreatic duct with 2% NaT as described previously elsewhere.[3,4] Briefly, adult male wild-type or *Trpv1*$^{-/-}$ C57BL/6 mice were anesthetized by intraperitoneal injection of a mixture of 87.5% ketamine/12.5% xylazine, and a midline laparotomy was used to expose the first portion of the duodenum. A puncture wound was made in the antimesenteric surface of the duodenum opposite the ampulla of Vater, and a 30G catheter attached with tubing to an infusion pump was passed through the puncture wound and then into the common bile duct via the ampulla of Vater. The infusion catheter was secured in the distal common bile duct distal to the entrance of the pancreatic duct with a ligature, and the bile duct near the liver was occluded with a bulldog clamp. The inflammatory infusate consisted of 2% NaT in 0.9% NaCl pumped into the pancreatic duct at a rate of 5 μL/min for 10 minutes with a syringe pump. Methylene blue (1%) was included in the infusate to allow identification of leakage from the duct lumen. After 10 minutes, the catheter, ligature, and bulldog clamp were removed, and the duodenotomy was closed using a purse-string suture. The laparotomy was closed in two layers, and analgesia was achieved by subcutaneous injection of buprenorphine hydrochloride at a dose of 50 μg/kg. The animals were given free access to food and water upon recovery. The mice were killed 24 hours after surgery by CO_2 asphyxiation and then were weighed. The pancreas was removed and weighed; a portion was frozen at -80°C for later myeloperoxidase (MPO) assay, and a separate portion was fixed overnight at 4°C in 10% formalin for the histopathologic analysis. Mixed arteriovenous blood was also collected by decapitation for serum amylase measurement.

Acute pancreatitis was also induced by secretagogue hyperstimulation using 6 hourly subcutaneous injections of caerulein at a dose of 50 μg/kg, as previously described elsewhere.[7,12] The mice were killed 1 hour after the last caerulein injection by CO_2 asphyxiation, and the tissues collected as described previously.

The pharmacologic treatments tested for their ability to inhibit NaT-induced acute pancreatitis included RTX, an excitotoxin that desensitizes TRPV1 when used at high concentrations,[13] and MK886, a 5-lipoxygenase-activating peptide (FLAP) inhibitor that inhibits LTB_4 biosynthesis by blocking 5-LO activity.[14] RTX was dissolved in ethanol and administered in 97.5% saline/2.5% ethanol either alone (in control experiments) or together with 2% NaT in 0.9% NaCl at a concentration of 14 μg/mL in the pancreatic ductal infusate by retrograde infusion at 5 μL/min for 10 minutes. RTX was given by intraductal infusion because this route of delivery is effective in desensitizing TRPV1.[15] The same RTX dose was demonstrated to be efficacious in inhibiting acute pancreatitis in rats caused by pancreatic ductal infusion of low pH endoscopic retrograde cholangiopancreatography contrast solution.[15] MK886 was administered as a pretreatment 1 hour before surgery by intraperitoneal injection at a dose of 10 mg/kg dissolved in 2% ethanol/2% Tween 80/96% sterile 0.9% NaCl. Control mice were treated similarly with the vehicle alone. This dose of MK886 has previously been shown to inhibit pancreatic duct ligation-induced acute pancreatitis in rats.[16]

Serum Amylase Activity

The serum amylase concentration was measured as previously described elsewhere[17] except that Phadebas amylase test tablets (Magle Life Sciences, Cambridge, MA) were used as substrate instead of Procion Yellow starch.

Myeloperoxidase Activity

We measured the tissue activity of MPO, an enzyme produced by neutrophils and used as a marker of inflammation associated with neutrophil infiltration, as previously described elsewhere using the substrate tetramethylbenzidine.[18,19] The