Gastroenterology 2018;154:689–703

Mitochondrial Dysfunction, Through Impaired Autophagy, Leads to Endoplasmic Reticulum Stress, Deregulated Lipid Metabolism, and Pancreatitis in Animal Models

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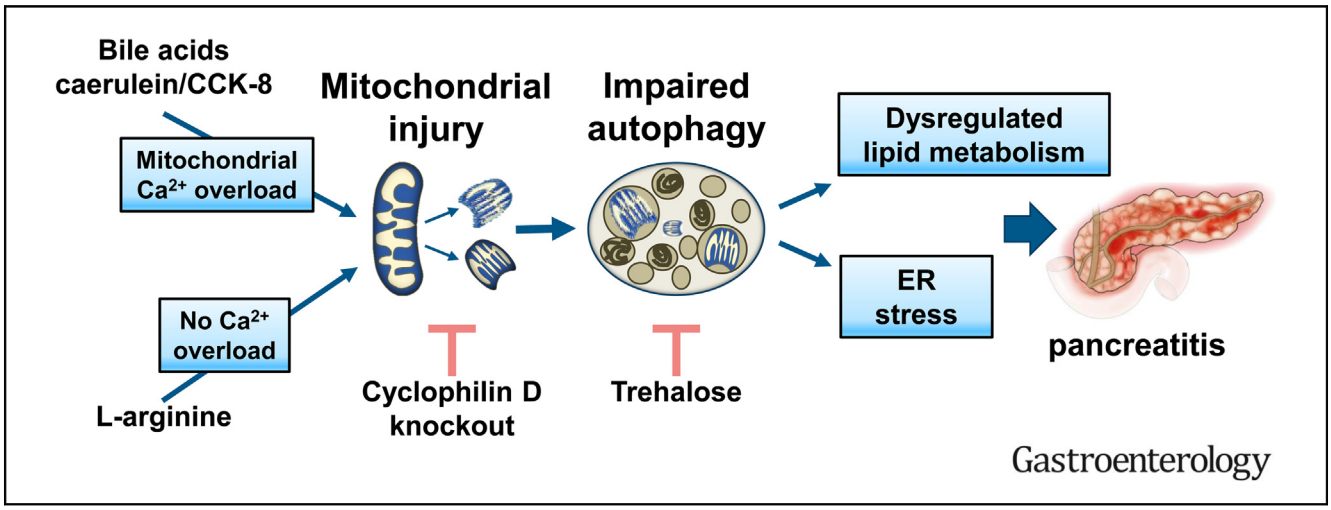

BACKGROUND & AIMS: Little is known about the signaling pathways that initiate and promote acute pancreatitis (AP). The pathogenesis of AP has been associated with abnormal increases in cytosolic Ca^{2+}, mitochondrial dysfunction, impaired autophagy, and endoplasmic reticulum (ER) stress. We analyzed the mechanisms of these dysfunctions and their relationships, and how these contribute to development of AP in mice and rats. **METHODS:** Pancreatitis was induced in C57BL/6J mice (control) and mice deficient in peptidylprolyl isomerase D (cyclophilin D, encoded by *Ppid*) by administration of L-arginine (also in rats), caerulein, bile acid, or an AP-inducing diet. Parameters of pancreatitis, mitochondrial function, autophagy, ER stress, and lipid metabolism were measured in pancreatic tissue, acinar cells, and isolated mitochondria. Some mice with AP were given trehalose to enhance autophagic efficiency. Human pancreatitis tissues were analyzed by immunofluorescence. **RESULTS:** Mitochondrial dysfunction in pancreas of mice with AP was induced by either mitochondrial Ca^{2+} overload or through a Ca^{2+} overload-independent pathway that involved reduced activity of ATP synthase (80% inhibition in pancreatic mitochondria isolated from rats or mice given L-arginine). Both pathways were mediated by cyclophilin D and led to mitochondrial depolarization and fragmentation. Mitochondrial dysfunction caused pancreatic ER stress, impaired autophagy, and deregulation of lipid metabolism. These pathologic responses were abrogated in cyclophilin D-knockout mice. Administration of trehalose largely prevented trypsinogen activation, necrosis, and other parameters of pancreatic injury in mice with L-arginine AP. Tissues from patients with pancreatitis had markers of mitochondrial damage and impaired autophagy, compared with normal pancreas. **CONCLUSIONS:** In different animal models, we find a central role for mitochondrial dysfunction, and for impaired autophagy as its principal downstream effector, in development of AP. In particular, the pathway involving enhanced interaction of cyclophilin D with ATP synthase mediates

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Acinar cell mitochondrial dysfunction and impaired autophagy are implicated in the pathogenesis of pancreatitis, a disease with no effective treatment. Mechanisms of these dysfunctions, their interrelations, and links to acute pancreatitis pathology are poorly understood.

NEW FINDINGS

Mitochondrial dysfunction in experimental acute pancreatitis is mediated by cyclophilin D and involves Ca^{2+}-overload-dependent or -independent pathways. The latter mediates L-arginine pancreatitis, a severe model with unknown pathogenesis. Mitochondrial dysfunction causes impaired autophagy, ER stress and deregulated lipid metabolism, which are normalized by cyclophilin D knockout.

LIMITATIONS

There is limited information on the mechanisms of mitochondrial dysfunction and impaired autophagy in human disease.

IMPACT

Restoration of mitochondrial function and/or efficient autophagy greatly ameliorates pancreatitis in mouse models, and thus offers potential treatment approaches for this disease.

L-arginine–induced pancreatitis, a model of severe AP the pathogenesis of which has remained unknown. Strategies to restore mitochondrial and/or autophagic function might be developed for treatment of AP.

Keywords: Pancreas; Inflammatory Response; Acinar Cell; Lamellar Bodies.

The pathogenic mechanism of acute pancreatitis (AP), a common and sometimes fatal disease, is incompletely understood, and no specific/effective treatment is available.[1,2] Because of limited access to human tissue, the knowledge on molecular/cellular pathways initiating and driving pancreatitis comes mainly from experimental models that appear to reproduce key responses of human disease.[3] There are significant gaps in our understanding of these pathways, in particular the role of mitochondrial dysfunction. It has been recently shown[4] that persistent opening of permeability transition pore (PTP) caused by mitochondrial Ca^{2+} overload is an early event in experimental AP models associated with pathologic (global and sustained) increases in free cytosolic Ca^{2+} ($[Ca^{2+}]i$), such as those induced by caerulein (CER-AP) or pancreatic ductal infusion of taurolithocholic acid sulfate (TLCS-AP). PTP is a non-specific channel traversing both the outer and inner mitochondrial membranes, persistent opening of which causes depolarization and, ultimately, adenosine triphosphate (ATP) drop.[5] In those models, PTP blockade by genetic or pharmacologic knockdown of its major regulator peptidylprolyl isomerase D (cyclophilin D) markedly ameliorated disease.[4] However, it remains unknown whether mitochondrial dysfunction drives AP in models where massive increases in $[Ca^{2+}]i$ have not been reported (such as that induced by L-arginine (Arg-AP) or by genetic modifications[6]); and if so, what the underlying mechanisms are.

The paradigm on PTP molecular nature has dramatically changed, with recent findings showing that the 15-subunit ATP synthase is a central PTP component.[5] Under certain conditions, ATP synthase undergoes cyclophilin D-dependent conformational transition, forming the PTP channel. The detailed mechanism is a matter of intense investigation, but it is believed that the multisubunit structure of ATP synthase restricts the switch to PTP conformation and this restriction is removed by cyclophilin D.[5,7] Whether such mechanism operates in disease has not been shown.

Very little is known about the mechanisms linking mitochondrial dysfunction to AP responses. Recent studies revealed, in particular, that impaired autophagy is a major pathologic event prominent in both experimental and human pancreatitis.[8,9] It is implicated in disease initiation because genetic modifications to inhibit or impair autophagy cause pancreatitis.[10–12] Endoplasmic reticulum (ER) stress is another pathway believed to be involved in AP.[13,14] However, whether disordering of pancreatic autophagy or activation of ER stress are linked to mitochondrial dysfunction remains unclear. Furthermore, it is not known whether normalizing these pathways alleviates AP. Hypertriglyceridemia is an established risk factor for human pancreatitis[15,16]; however, it is not known whether pancreatitis alters acinar cell lipid metabolism and whether mitochondria and/or autophagy play a role in these alterations.

To examine these issues, we primarily utilized the Arg-AP model. Although this noninvasive model of severe AP was introduced decades ago and is being increasingly used,[17,18] its pathogenic mechanism remains largely unknown.

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Abbreviations used in this paper: **ADP, adenosine diphosphate; AP, acute pancreatitis; Arg, L-arginine; Arg-AP, L-arginine-induced acute pancreatitis; ATP, adenosine triphosphate; CatB, cathepsin B; CCK, cholecystokinin-8; CDE-AP, pancreatitis induced with choline deficient, ethionine-supplemented diet; CER-AP, cerulein-induced acute pancreatitis; CypD, cyclophilin D; $[Ca^{2+}]i$, free cytosolic Ca^{2+} concentration; $\Delta\Psi m$, mitochondrial membrane potential; EM, electron microscopy; EMSA, electrophoretic mobility shift assay; ER, endoplasmic reticulum; FFA, free fatty acid; GC-MS, gas chromatography-mass spectrometry; IB, immunoblot; IF, immunofluorescence; IHC, immunohistochemistry; i.p., intraperitoneal; KO, knockout; LB, lamellar body; LC-MS, liquid chromatography-mass spectrometry; LD, lipid droplet; L-MMNA, N^G-monomethyl-L-arginine acetate; MPO, myeloperoxidase; PLIN, perilipin; PTP, permeability transition pore; PUFA, polyunsaturated fatty acid; SEM, standard error of the mean; TAG, triacylglycerol; TLCS-AP, pancreatitis induced by taurolithocholic acid sulfate infusion; wt, wild type.**

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0016-5085/$36.00
https://doi.org/10.1053/j.gastro.2017.10.012