Calcium signaling, mitochondria and acute pancreatitis: avenues for therapy

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1. The role of the pancreatic acinar cell in acute pancreatitis:

Pancreatic necrosis, systemic inflammatory response syndrome, multiple organ failure and sepsis are characteristic of severe acute pancreatitis, which results in death of one in four patients and is without specific drug therapy (59, 62). As the pancreatic acinar cell is an initial site of injury (41, 59), commonly initiated by bile or ethanol excess, investigation of its behaviour in response to toxins that induce acute pancreatitis may determine critical mechanisms and importantly identify new drug targets. In view of the critical role of calcium signaling in normal stimulus-secretion and stimulus-metabolism coupling, and the long known toxicity of raised intracellular calcium, we proposed the hypothesis that prolonged elevations of cytosolic calcium is the key trigger of acute pancreatitis (83). Since that proposal over 20 years ago, increasing evidence has confirmed that sustained elevation of the cytosolic calcium concentration ($[Ca^{2+}]_C$) is a critical trigger for pancreatic acinar cell injury and necrosis that depends on store-operated calcium entry (SOCE) (12, 24, 36, 39, 63, 84).

2. The critical role of calcium entry in acinar cell injury:

Intracellular Ca^{2+} signals control normal secretion from pancreatic acinar cells but can become a critical trigger in pathogenesis. Physiological concentrations of acetylcholine (ACh) and cholecystokinin (CCK) generate repetitive elevations in $[Ca^{2+}]_C$ within the cellular apical pole that elicit stimulus-metabolism coupling to generate adenosine triphosphate (ATP) from mitochondria and stimulus-secretion coupling to initiate exocytosis (61). Intermittently, global extension of short-lived signals throughout the cell are necessary for nuclear signalling contributing to transcription and translation (61). Elevations of $[Ca^{2+}]_C$ are buffered in mitochondria, notably those surrounding the apical pole, with subsequent reuptake into the endoplasmic reticulum (ER) by sarcoER Ca^{2+} ATPase (SERCA) pumps and extrusion by plasma membrane calcium pump (PMCA) **(Figure 1)**. In contrast, toxins such as bile acids (81), oxidative (70) and non-oxidative metabolites (12, 34) of ethanol and CCK hyperstimulation (10, 53) each elicit abnormal elevations of $[Ca^{2+}]_C$ that are global and sustained. The abnormal elevations induce premature activation of intracellular enzymes, mitochondrial dysfunction, impaired autophagy,

vacuolization and necrosis, all of which contribute to the pathogenesis of acute pancreatitis (11). Maintenance of these abnormal elevations depends on continued emptying of the ER Ca^{2+} store and activation of store-operated Ca^{2+} entry (SOCE) and Ca^{2+}-release activated Ca^{2+} currents (CRAC) to replenish the ER store (24, 84). Ca^{2+} chelation prevents zymogen activation and vacuolization through attenuation of Ca^{2+} overload in acinar cells *in vitro* (63, 67) and ameliorates the severity of acute pancreatitis *in vivo* (49).

Excessive Ca^{2+} release from intracellular stores occurs predominantly via inositol 1,4,5-trisphosphate receptor (IP_3R) Ca^{2+} channels (26). The pancreatic acinar cell expresses all three subtypes of the IP_3R in the apical region, close to the luminal membrane (21, 45, 46) but IP_3R types

2 and 3 are predominantly responsible for physiological Ca^{2+} signaling and enzyme secretion (21). Stimuli such as CCK (7) the bile acid taurolithocholic acid 3-sulphate (TLCS) (23, 79), alcohol (25) and fatty acid ethyl esters (FAEEs) (12, 26) cause intracellular Ca^{2+} release in pancreatic acinar cells primarily via IP_3Rs, an effect inhibited by double knockout of IP_3R types 2 and 3 (21) or by caffeine (26, 53).

Since the discovery of the calcium entry channel ORAI1, ORAI1 has been shown to be the principal SOCE channel in the pancreatic acinar cell (45), opening of which is coordinated by stromal interaction molecules (STIM1 and STIM2), following decreases in ER calcium store concentrations (16, 24, 45, 50).
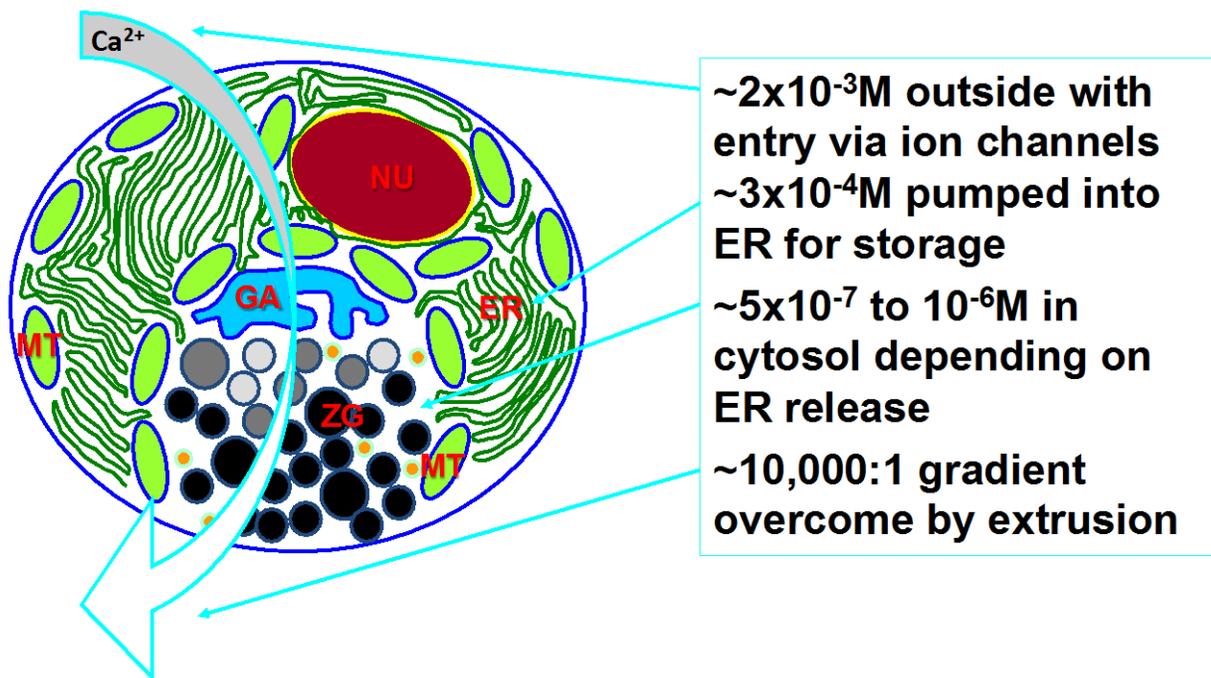

Ca²⁺

~2x10⁻³M outside with entry via ion channels

~3x10⁻⁴M pumped into ER for storage

~5x10⁻⁷ to 10⁻⁶M in cytosol depending on ER release

~10,000:1 gradient overcome by extrusion

Figure 1. Ca^{2+} signaling in the pancreatic acinar cell depends on tight control of concentrations across the plasma membrane and within subcellular organelles. In resting conditions [Ca2+]c is ~10,000 fold lower than outside the cell, with Ca^{2+} stored mainly in the ER. Stimulus-secretion coupling operates by secretagogue-elicited GPCR activation to release second messengers that bind to inositol trisphosphate and ryanodine receptors on the ER, through which Ca^{2+} is released into the cytosol and mitochondria, initiating ATP production that provides energy for secretion. Ca^{2+} is cleared by reuptake into the ER and extrusion from the cell; the Ca2+ extruded is replenished via puncta forming between the ER and plasma membrane in response to low ER Ca2+ levels, allowing entry via ORAI channels. Pancreatitis toxins induce excessive ER Ca^{2+} release, initiating a vicious circle that overwhelms the cell (ER: endoplasmic reticulum; GA: Golgi apparatus; MT: mitochondria; NU: nucleus; ZG: zymogen granules).

GSK-7975A and CM_128 have been developed independently by GlaxoSmithKline (16, 24, 64) and CalciMedica (84) respectively to block ORAI1 channels, although only CM_128 continues towards clinical development. GSK-7975A inhibits SOCE induced by thapsigargin in isolated murine pancreatic acinar cells over the range 1-50 μM (IC_{50} ~3.4 μM) (24), inhibits endocytic vacuole formation (78) and reduces necrosis induced by toxins that cause acute pancreatitis (24, 78, 84). CM_128 is a new molecular entity, the effects of which we have recently confirmed to be similar (84). We have also shown that ORAI inhibition inhibits SOCE and necrosis in human pancreatic acinar cells, and found ORAI inhibition to reduce markedly the severity of multiple models of experimental acute pancreatitis.

Genetic knockout of the transient receptor potential canonical (TRPC) 3 channel (38), a non-selective cation channel regulated in part by STIM1 via TRPC1 (42), results in ~50% reduction of *in vivo* serum amylase elevation and edema formation induced by four injections of cerulein (38). These experiments supported a role for SOCE in acute pancreatitis, but in a single, mild model with few parameters of response.

As indicated we have defined the concentration-dependent inhibitory effects of GSK-7975A and CM_128 on SOCE and necrosis in murine and human pancreatic acinar cells induced by TLCS (60, 79) or CCK-8 (38, 63). The effects of CM_128 on ORAI1 were confirmed by examination of its effect on Ca^{2+} release-activated Ca^{2+} currents (I_{CRAC}) (16, 24, 50), in ORAI1/STIM1-transfected HEK 293 cells (16). GSK-7975A was given at selected doses *in vivo* after induction of acute pancreatitis with TLCS (TLCS-AP) (40), seven injections of cerulein (CER-AP) (43) or ethanol and palmitoleic acid (FAEE-AP) (34). Since GSK-7975A markedly reduced all parameters of pathobiologic response in a dose-dependent manner, a high dose of GSK-7975A and separately CM_128 was begun at two different time points after disease induction to determine the effect of early versus late drug administration. Drug administration begun one hour after disease induction was highly effective in reducing parameters of the pathobiologic response (84), significantly more so than when begun six hours after disease induction, in all models. These data provide thorough preclinical validation for ORAI channel inhibition as a potential early treatment for acute pancreatitis.

We found GSK-7975A and the new molecular entity CM_128 to inhibit toxin-induced SOCE into murine and human pancreatic acinar cells in a concentration-dependent manner, exceeding >90% block of relative control values in some protocols (84). We also found both GSK-7975A and CM_128 to significantly reduce necrotic cell death pathway activation in murine and human pancreatic acinar cells exposed to TLCS, which induces acute pancreatitis *in vivo* (40, 43). While effects of GSK-7975A have been described on thapsigargin- and FAEE-induced murine pancreatic acinar SOCE (24), our studies found GSK-7975A to have a similarly critical effect on TLCS- and CCK-induced murine pancreatic acinar SOCE, as well as thapsigargin-induced human pancreatic acinar SOCE and TLCS-induced human pancreatic acinar necrotic cell death pathway activation (84). CM_128 showed higher potency (IC_{50} ~0.1 μM from ORAI1/STIM1-transfected HEK 293 cell patch clamp data), and unlike GSK-7975A, no loss of efficacy at high doses. Comprehensive *in vivo* evaluation using three diverse, clinically representative models of acute pancreatitis (84) with prior pharmacokinetic assessment demonstrated the validity of SOCE inhibition as a therapeutic approach. Thus administration of either compound within one hour following disease induction was markedly effective across a representative range of local and systemic biochemical, immunological and histological disease responses. These data provide robust confirmation of the hypothesis that cytosolic Ca^{2+} overload is a critical trigger of acute pancreatitis (83).

Further confirmation of the role of cytosolic Ca^{2+} overload in acute pancreatitis has come from our

work with xanthines (35). We defined the inhibitory effects of methylxanthines on IP_3R-mediated Ca^{2+} release from the pancreatic acinar ER store into the cytosol and potential application in acute pancreatitis. It has been shown that caffeine inhibits IP_3Rs as well as IP_3 production in a concentration dependent manner (75). We found that inhibition of IP_3R-mediated Ca^{2+} release is attributable at least in part to an action on the IP_3R, since xanthines inhibited IP_3R-mediated Ca^{2+} release elicited by uncaged IP_3 (35). Caffeine, theophylline and paraxanthine prevented physiological Ca^{2+} signaling and toxic elevations of $[Ca^{2+}]_C$ induced by agents (CCK and TLCS) that cause acute pancreatitis, in a concentration dependent manner (500 µM to 10 mM), also inhibiting falls in mitochondrial membrane potential ($\Delta\Psi_M$) and necrotic cell death pathway activation. An inhibitory action on phosphodiesterase (PDE) preventing cAMP/cGMP degradation could not account for the effects on toxic $[Ca^{2+}]_C$ overload, since additional cAMP/cGMP did not prevent these. Extending these findings *in vivo*, caffeine significantly reduced the severity of multiple, diverse models of acute pancreatitis (35). The combined concentrations of di- and trimethylxanthines after the 25 mg/kg caffeine protocol were within the range over which effects on both IP_3R-mediated Ca^{2+} release and toxic elevations of $[Ca^{2+}]_C$ were identified. Despite the half-life of caffeine in mice of ~60 min (4), the combined peak concentrations of di- and trimethylxanthines with 25 mg/kg caffeine regimen (7 injections) were >2 mM, and serum caffeine was >400 µM 6 h after last caffeine injection. Following similar protocols of 25 mg/kg theophylline or paraxanthine, concentrations were far below the effective range on IP_3Rs but within the effective range on PDE (approaching 100 µM 10 min after the last dimethylxanthine injection) (20), and no protective effects on *in vivo* acute pancreatitis were seen. Since pancreatic cellular injury initiates and determines severity in acute pancreatitis, the protective effect of caffeine on

acute pancreatitis is likely to have been mediated by inhibition of IP_3R-mediated Ca^{2+} release.

3. The effects of calcium elevation in mitochondria:

The pancreatic acinar cell typifies non-excitable exocrine cells with a high secretory turnover that is heavily dependent on mitochondrial production of ATP (61). While zymogen activation has long been considered the principle mechanism of injury (41, 59), mitochondrial dysfunction has been increasingly implicated (12, 34, 44, 68, 70, 74, 80), presumed consequent upon intracellular Ca^{2+} overload induced by toxins that include bile acids and ethanol metabolites (5, 12, 34). Mitochondrial uptake of Ca^{2+} drives normal cellular bioenergetics, but high calcium loads induce increasingly drastic responses culminating in necrosis (30). Mitochondrial matrix Ca^{2+} overload leads to opening of the mitochondrial permeability transition pore (MPTP), a non-specific channel that forms in the inner mitochondrial membrane allowing passage of particles under 1500 Da, causing loss of $\Delta\psi_m$ essential to ATP production (30); recent evidence implicates F_0F_1 ATP synthase in MPTP formation (1, 27). MPTP opening is physiological in low conductance mode releasing Ca^{2+} and reactive oxygen species (ROS) to match metabolism with workload (17, 18), but pathological in high conductance mode compromising ATP production and inducing cell death (30); both functions are regulated by the mitochondrial matrix protein peptidyl-prolyl *cis-trans* isomerase (PPI, cyclophilin) D (also known as cyclophilin F) (76).

Our work has demonstrated that MPTP opening is critical to experimental acute pancreatitis, mediating impaired ATP production, defective autophagy, zymogen activation, inflammatory responses and necrosis (52), all features of acute pancreatitis at molecular, cellular and whole organism levels (59). We have established the general significance of MPTP opening as a central mechanism in the pathogenesis of acute

pancreatitis, and the primary role in this process of Ca^{2+} overload. Patch clamp data show how tight control of cytosolic Ca^{2+} elevations essential to normal stimulus-secretion coupling by IP_3Rs and ryanodine receptors (RyRs) (61) is lost in Wt but maintained in *Ppif*^{-/-} pancreatic acinar cells, which lack functional MPTP, preserve ATP supply and clear Ca^{2+} more effectively. Coupling of ER IP_3Rs and RyRs with outer mitochondrial membranes tightly localizes high Ca^{2+} concentrations (65), but may expose mitochondria to abnormal Ca^{2+} release, despite modulation by Bcl-2 family proteins (74). We have shown that pancreatitis toxins cause abnormal release of Ca^{2+} via IP_3Rs and RyRs that overloads pancreatic acinar mitochondria (52), which are markedly sensitive to Ca^{2+} signals (58). The mitochondrial Ca^{2+} overload induces high conductance MPTP opening and dissipates $\Delta\psi_m$, initiating collapse of ATP production, diminished Ca^{2+} clearance, PGAM5 (phosphoglycerate mutase family member 5, a mitochondrial protein phosphatase) activation and subsequent necrosis (52). Importantly for a disease without specific treatment, pharmacological MPTP inhibition (55, 84) administered after acute pancreatitis induction came close to preventing all injury, notably in the clinically relevant TLCS-AP.

For more than a century following an original postulate by Chiari (8), acute pancreatitis has been viewed as an autodigestive disease consequent on pathological zymogen activation (22, 29, 36, 41, 66). In experimental acute pancreatitis zymogens are activated inside acinar cells within minutes of toxin exposure (41, 47, 59, 63), which we have shown to result from induction of the MPTP, caused by and contributing to Ca^{2+} overload. Sustained Ca^{2+} overload may activate degradative calpains, phospholipases or other enzymes (76) and damage zymogen granules, inducing autophagic (47) and/or endolysosomal (71) responses that activate digestive enzymes. Such activation was not completely prevented by MPTP inhibition, however, this was likely from global cytosolic Ca^{2+} overload that was seen to be more effectively cleared in *Ppif*^{-/-} cells, without which overload no enzyme activation occurs (63). Nevertheless, intracellular expression of trypsin *per se* without mitochondrial injury leads to apoptotic not necrotic pathway activation (22) and trypsinogen activation does not appear necessary for either local or systemic inflammation (13); knockout of cathepsin B greatly reduces trypsinogen activation with little effect on serum interleukin-6 or lung injury (29). Hereditary pancreatitis caused by cationic trypsinogen gene mutations rarely features clinically significant pancreatic necrosis (33, 85); further, systemic protease inhibition has had little success as a clinical strategy (59), suggesting that while zymogen activation contributes, it is not the critical driver of acute pancreatitis. Our work, however, shows that MPTP opening triggers defective autophagy, while inhibition of MPTP opening preserved ATP supply, increased the efficiency of autophagy and decreased zymogen activation. Together with major effects of MPTP opening on PGAM5 activation that implements necrosis (69, 82), and on local and systemic inflammatory responses, these findings now place mitochondrial injury centrally in acute pancreatitis.

Our data show that in pancreatic acinar cells IP_3Rs and RyRs are vulnerable to specific toxins that markedly increase their Ca^{2+} channel open-state probabilities (52). Toxic transformation of Ca^{2+} channel function induced pancreatic acinar cell necrosis through Ca^{2+}-dependent formation of the MPTP, with diminished ATP production the critical consequence. Toxic transformation by different toxins was specific to different second messengers, identifying potential for a variety of deleterious effects. ATP deficiency may be further exacerbated by fatty acids released on hydrolysis of FAEEs or triglycerides (56), which may inhibit beta-oxidation (12). Without sufficient ATP, cytosolic Ca^{2+} overload produces a vicious circle in which high affinity, low capacity SERCA and PMCA pump clearance of cytosolic Ca^{2+} is impaired, further mitochondrial injury sustained, and necrotic cell death accelerated (5, 12).

Although the toxicity of cytosolic Ca^{2+} overload depends on Ca^{2+} store refilling from outside the cell (38, 63), specific second messenger receptor blockade demonstrated Ca^{2+} overload to be due completely to release from their Ca^{2+} channels (52), not direct effects of toxins on Ca^{2+} entry or extrusion.

Whereas the vast majority of previous studies undertaken to determine mechanisms and/or new targets in acute pancreatitis have used only one model, we have used four models (52) that are broadly representative of a range of etiologies, including biliary (TLCS-AP), hyperstimulation (CER-AP), ethanolic (FAEE-AP) and amino acid-induced (CDE-AP) (59, 72). Our findings in experimental acute pancreatitis are entirely consistent with those made in isolated mitochondria and cells, identifying a generalized mechanism of pancreatic injury and necrosis, confirmed in murine and human pancreatic acinar cells, pancreas lobules and tissue slices. Pancreatic necrosis drives the inflammasome (32), which can be induced by MPTP opening (54) and is part of the systemic inflammatory response contributing to multiple organ failure (62). Further pancreatic injury is driven through tumor necrosis factor receptor activation that also promotes MPTP opening (31) and calcium deregulation, activating calcineurin and calcineurin-dependent transcription factor nuclear factor of activated T cells (51).

4. Therapeutic avenues for acute pancreatitis:

Our novel human data support the potential applicability of SOCE inhibition as a treatment for clinical acute pancreatitis **(Figure 2)**. Both GSK-7975A and CM_128 blocked SOCE promptly, shown to result in complete block of human ORAI1 by CM_128 (84). While an action on other ORAI channels cannot be excluded and could be desirable, ORAI1 is the primary channel for SOCE into pancreatic acinar cells (24, 45), blocked by both compounds. ORAI channels also contribute to inflammatory cell responses, including neutrophil migration and activation (3); inhibition of innate immune responses significantly reduces the severity of experimental acute pancreatitis (28), thus there may be a contribution from ORAI inhibition of immune cells. Nevertheless while knockout of ORAI1/STIM1 SOCE inhibits neutrophil functions, it does not prevent all (3), so the primary contribution of ORAI blockade in our experiments is likely to have been in the pancreas. Further, since SOCE inhibition for clinical acute pancreatitis would necessarily be short-term, inhibition of the adaptive immune system (3) would also be short-term. ORAI blockade has less effect on other cell types in which ORAI channels have a less prominent role, such as electrically excitable cells in which other ion channels, e.g. non-selective cation channels, have a larger role in Ca^{2+} entry (9). Non-selective cation channels, however, permit limited SOCE into pancreatic acinar cells (24, 38) that could sustain essential Ca^{2+} entry (9). Without such Ca^{2+} entry, continued activation of the plasma membrane Ca^{2+}-ATPase pump upon secretagogue- or toxin-mediated release of Ca^{2+} from intracellular stores could deplete these stores to deleterious levels, inducing or exacerbating ER stress (48).

Both ORAI inhibitory compounds were administered after disease induction to model treatment of clinical acute pancreatitis, but delay in administration of either compound to six hours after disease induction resulted in diminished efficacy, dependent on the endpoint measured and the model employed (84). While biological time courses including that of acute pancreatitis are longer in humans than mice (15, 43, 59, 62), with pancreatic necrosis typically detected within days rather than hours (73), human pancreatic acinar necrotic cell death pathway activation may begin in clinical acute pancreatitis at an early stage after disease onset, shown here in mouse models within six hours of onset.
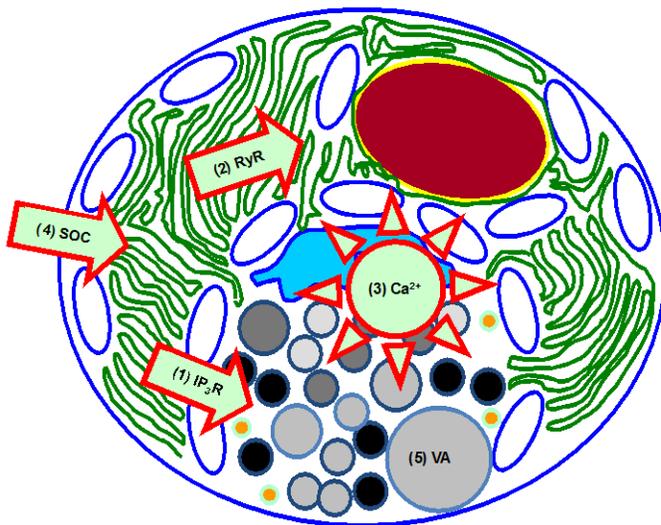
(1) Pancreatitis toxins open IP$_3$Rs and (2) RyRs causing (3) Ca^{2+} release; (4) ER Ca^{2+} depletion results in STIM1- and STIM2-mediated entry through ORAI channels; (5) Mitochondrial Ca^{2+} overload impairs ATP production, disorders autophagy, inducing vacuole (VA) formation

Figure 2. Abnormal Ca^{2+} signaling in the pancreatic acinar cell initiated by pancreatitis toxins (e.g. bile acids, FAEEs, hyperstimulation) causes injury dependent on continued store-operated Ca^{2+} (SOC) entry via ORAI channels. As a consequence mitochondria are overloaded with Ca^{2+}, failing to produce adequate ATP to clear the Ca^{2+} and protect the cell. Autophagy is defective and vacuoles develop presaging death of the cell. Ca^{2+} entry blockade prevents these events, avoiding prolonged, global cytosolic Ca^{2+} overload. This was first demonstrated by removal of Ca^{2+} from the external medium surrounding isolated cells and has since been shown using ORAI blockers applied to isolated human and murine pancreatic acinar cells. These findings have since been extended into three murine models of experimental acute pancreatitis, in which this strategy of Ca^{2+} entry inhibition with ORAI blockers has been demonstrated to be highly effective.

Door-to-needle times of less than 60 minutes are established guidelines for patients with acute myocardial infarction (30 min) (57) and acute ischemic stroke (60 min) (37), making every second count, with national and international quality improvement initiatives underway towards fully achieving these (19).

Although pancreatic necrosis has a less rapid time course and is characteristically not the result of major arterial occlusion (59), the translational implication of our work is that door-to-needle time is an important issue in administration of any treatment for acute pancreatitis that targets the pathogenesis of pancreatic injury, which drives the disease. Previously clinical trials of treatments for acute pancreatitis have 'enriched' recruitment with patients predicted to have severe disease

(often with recruitment up to 72 h after admission) (77), which delays initiation of therapy. Furthermore, the expansion of disease categories from the original Atlanta Classification (mild and severe) (6) into the revised Atlanta (mild, moderate and severe) (2) and Determinants-Based (mild, moderate, severe, critical) (14) classification, further complicates patient selection from among these potentially overlapping sub-groups. To minimize door-to-needle time a quicker and more accurate approach to the selection of patients is required for trials of any therapy, such as that offered here with ORAI inhibition by CM_128, a novel molecular entity currently undergoing preclinical toxicological evaluation prior to phase I trials.

With respect to inhibition of Ca^{2+} release within the pancreatic acinar cell rather than Ca^{2+} entry into the cell, it is important to note that in our studies high doses of caffeine were required to reduce the severity of experimental acute pancreatitis. The most effective 25 mg/kg regimen extended into toxicity, indicative of a very narrow therapeutic index. At this dose the number of hourly injections had to be reduced from seven to two in FAEE-AP to avoid mortality; in CER-AP, 50 mg/kg resulted in caffeine intoxication syndrome, although at 25 mg/kg no visible side effects were observed. In humans even 10 mg/kg caffeine would be likely to induce caffeine intoxication, with florid neuro-excitotoxic and other undesirable side effects (20). There is marked individual variability in caffeine metabolism and pharmacokinetics (20); since the half-life in humans typically ranges from three to seven hours, repeated high doses would be hazardous, unless rapid therapeutic monitoring were to be possible. Nevertheless our study has demonstrated proof of principle that caffeine causes marked amelioration of experimental acute pancreatitis, largely through inhibition of IP3R-mediated signalling. Medicinal chemistry starting with the template of caffeine and/or other compounds that inhibit IP3R-mediated signalling could lead to more potent, selective and safer drug candidates for acute pancreatitis. This approach, however, might have effects on IP3R-mediated signalling in other cells, tissues and organs including the brain and other solid organs.

Our data from the cyclophilin D knockout and pharmacotherapy with Debio-025 (non-immunosuppressive derivative of cyclosporin A) or TRO40303 (in clinical development for other indications) show the potential for MPTP inhibition as an alternative strategy to ORAI inhibition **(Figure 3)**.
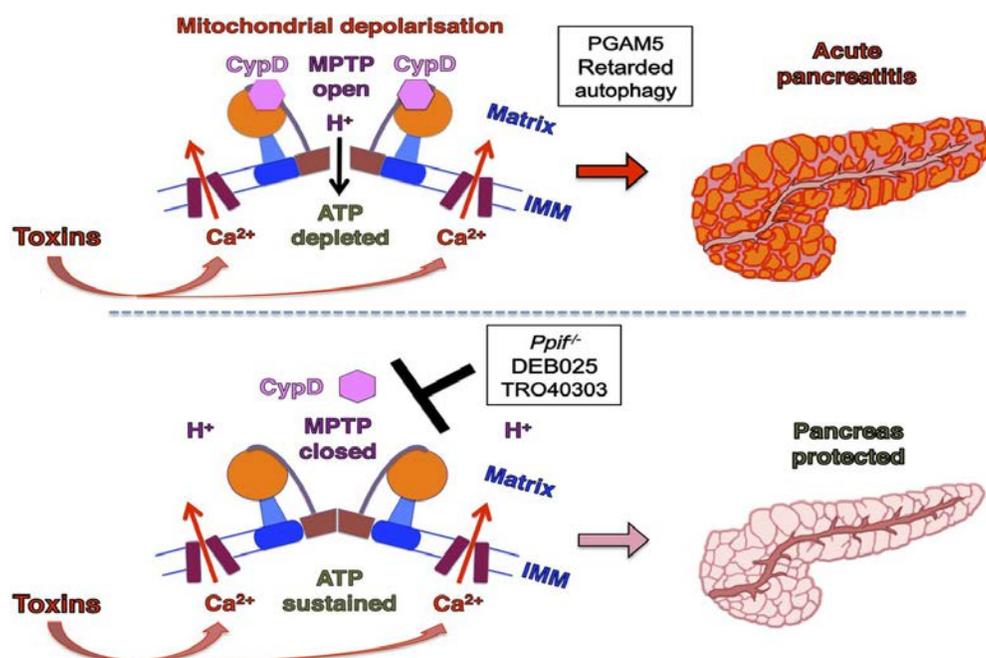

Figure 3. The MPTP plays a critical role in the development of acute pancreatitis. Pancreatitis toxins induce a sustained rise in [Ca2+]C that crosses the inner mitochondrial membrane (IMM) via the mitochondrial uniporter to enter the mitochondrial matrix. Consequent Ca^{2+}-cyclophilin D (CypD) activation promotes MPTP opening, causing mitochondrial depolarization and impaired ATP production, failure of Ca^{2+} clearance and cell injury. When MPTP opening is inhibited by genetic (Ppif^{-/-}) or pharmacological means (DEB025 or TR040303), mitochondrial membrane potential is preserved and ATP production sustained. This maintains cellular integrity to clear calcium more effectively and prevents the development of acute pancreatitis (lower panel) (Copyright © BMJ Publishing Group Ltd and British Society of Gastroenterology. All rights reserved: http://gut.bmj.com/content/65/8/1333.full).

The effect of this approach was remarkably effective in both isolated human and murine pancreatic acinar cells as well as in four models of experimental acute pancreatitis (52). The attractions of this approach are made stronger by the relative modest phenotype of the cyclophilin D knockout that is able to grow, develop and breed normally; there is an extensive range of pathologies that cyclophilin D knockout protects against, although capacity for exercise is reduced and there is some impact on memory in later life (17, 18). These effects are unlikely to be important in short-term administration as would be required in acute pancreatitis, and has not proven a problem in long-term administration of cyclosporin A.

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