Mitochondrial Permeability Transition Pore as a Therapeutic Target for Acute Pancreatitis

Thesis submitted in accordance with the requirements of
The University of Liverpool
for the degree of Doctor in Philosophy

by:

Muhammad Ahsan Javed

November 2017

Dedicated to

all patients who have suffered from acute pancreatitis

Table of contents

Abstract

Mitochondrial Permeability Transition Pore as a Therapeutic Target for Acute Pancreatitis

Muhammad Ahsan Javed

Introduction: Acute pancreatitis (AP) is frequently complicated by pancreatic necrosis, multiple organ failure, prolonged hospitalisation and death. There is no specific, licensed drug therapy for this disease to date. Opening of the mitochondrial permeability transition pore (MPTP) is a key event in the pathophysiology of AP and is therefore a valid drug target for its treatment. Cylophilin D (CypD, a mitochondrial matrix protein), is a key regulator of MPTP formation and opening of the latter is also modulated by a new drug TRO40303. The aim of this investigation is to explore the effects of newly identified CypD inhibitors and TRO4303 in both murine and human pancreatic acinar cells (PAC) in response to established pancreatic toxins as well as the severity of experimental acute pancreatitis (EAP). The study also provides a qualitative assessment of all published literature evaluating agents tested for treatment of EAP using a newly devised scoring system.

Methods: Confocal fluorescence microscopy of isolated PACs was used to evaluate the effects of MPTP inhibitors on mitochondrial function, calcium handling and cell fate in response to TLCS (taurolithocholate sulphate) and other pancreatic toxins - CCK (cholecystokinin) and POAEE (palmitoleic acid ethyl ester). In order to identify novel CypD inhibitors, computational modelling was undertaken. Compounds predicted to bind with CypD were screened using biophysical assay - surface plasmon resonance (SPR) - to evaluate binding affinity. Candidates with high affinity for CypD were subsequently evaluated in the aforementioned biological assays. Efficacy of TRO40303 in ameliorating the severity of EAP was tested in three different models.

Results: TRO40303 prevented loss of mitochondrial membrane potential ($\Delta\psi_m$) induced by TLCS, CCK and POAEE and improved Ca^{2+} clearance in PACs. TRO40303 reduced necrotic cell death pathway activation in response to TLCS in murine ($p<0.05$) as well as human PACs ($p<0.01$). Therapeutic administration of TRO40303 significantly reduced serum amylase, pancreatic trypsin, pancreatic and lung myeloperoxidase and histopathology scores in hyperstimulation, biliary and alcoholic EAP. Screening strategy of CypD inhibitors identified a novel small molecular inhibitor, AP-1A02, with a KD and Ki of 0.8 µM and 1.7 µM respectively. AP-1A02 protected $\Delta\psi_m$ and also significantly reduced necrotic cell death pathway activation in human as well as murine PACs in response to TLCS.

Conclusion: This work demonstrates that MPTP is a valid target for the treatment of AP. TRO40303 protects mitochondria, reduces necrotic cell death pathway activation and ameliorates the severity of EAP and is therefore a candidate drug for human AP. Further work needs to be undertaken to optimize AP-1A02 and develop other small molecule CypD inhibitors with the aim to develop it as a potential drug for AP.

Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the regulations of the University of Liverpool. The work is original except where indicated by special reference in the text and no part of the dissertation has been submitted for any other degree. The author in conjunction with his supervisors; Professor Robert Sutton, Professor Alexei Tepikin and Dr David Criddle, conducted all aspects of experimental design and planning for this study. The author performed all *in vitro* experiments included in this thesis independently. Experiments recording the severity of *in vivo* experimental pancreatitis were undertaken with a colleague, Li Wen and biophysical experiments were undertaken by a post-doctoral scientist Dr Muhammad Awais. Computational modelling of small molecule inhibitors of cyclophilin D was done in collaboration with Dr. Neil Berry – department of Medicinal Chemistry, University of Liverpool. Any views expressed in this thesis are those of the author and in no way represent those of the University of Liverpool. The thesis has not been presented to any other University for examination either in the United Kingdom or overseas.

List of Figures

List of Tables

List of Abbreviations

$\Delta\psi_m$	=	mitochondrial membrane potential
µM	=	micromolar
µl	=	microliter
ANT	=	adenine nucleotide translocase
AP	=	acute pancreatitis
ARDS	=	acute respiratory distress syndrome
ATP	=	adenine triphosphate
Ca^{2+}	=	cytosolic calcium
CCK	=	cholecystokinin
CCCP	=	carbonyl cyanide m-chlorophenylhydrazone
CER-AP	=	caerulein induced experimental acute pancreatitis
CsA	=	cyclosporin A
CypD	=	cyclophilin D
DMSO	=	dimethyl sulfoxide
EAP	=	experimental acute pancreatitis
ER	=	endoplasmic reticulum
ERCP	=	endoscopic retrograde cholangio-pancreatogram
FA	=	fatty acid
FAEE	=	fatty acid ethyl ester
IMM	=	inner mitochondrial membrane
IL	=	interleukin
MODS	=	multiple organ dysfunction syndrome
MPO	=	myeloperoxidase
MPTP	=	mitochondrial permeability transition pore
NAD(P)H	=	nicotenamide adenine dinucleotide phosphate
NFκB	=	nuclear factor κB
nM	=	nanomolar
OMM	=	outer mitochondrial membrane
PAC	=	pancreatic acinar cell
PI	=	propidium iodide
POA	=	palmitoleic acid
POAEE	=	palmitoleic acid ethyl ester
Ppif-/-	=	cyclophilin D knockout
PPIase	=	peptidylprolyl isomerase
ROS	=	reactive oxygen species
RCT	=	randomised controlled trial
SIRS	=	systemic inflammatory response syndrome
SPR	=	surface plasmon resonance
TLCS	=	tauro-lithocholate sulphate
TMRM	=	tetramethylrhodamine, methyl ester
TRO40303	=	3,5-Seco-4-nor-cholestan-5-one oxime-3-ol
TSPO	=	translocator protein
VDAC	=	voltage-dependent anion channel
ZG	=	zymogen granules

Acknowledgements

First of all, I would like to thank Professor Robert Sutton for his continual support, encouragement and teaching me how to ask relevant scientific questions and to devise strategies that would address these questions. His supervision throughout the course of my PhD has been outstanding. I would also like to thank Professor Alexei Tepikin and Dr David Criddle for providing me a home in Blue Block and their excellent scientific supervision.

There are many people in Blue Block and Pancreas Biomedical Research Unit who have really helped me over the course of my PhD and made my time here a thoroughly enjoyable experience. Dr Muhammad Awais has been a constant source of expert advice and scientific support and Dr Li Wen has been my partner in crime for the long cell death and *in vivo* experiments. Thanks to Svetlana for teaching me how to use the 510 confocal system, preparing high quality pancreatic acinar cells and Misha for always coming up with solutions to my scientific and statistical queries. I was fortunate to collaborate with colleagues in Medicinal Chemistry as well as Structural Biology during this project and would like to acknowledge the help and support provided by; Dr Neil Berry, Professor Paul O' Neil, Professor Liu Lian and Dr Emma Shore. I would also like to thank Dr. Sophie Schaller for establishing a strong collaboration with TROPHOS and supplying TRO40303. I was privileged to obtain funding from CORE as well as RCS England and really appreciate the financial support provided by these prestigious organisations.

I would like to thank my wonderful wife Sarah who has always been there for me, for her constant support and patience. To my beautiful daughter Fatima (eight year old) who understood that 'daddy has a big assignment to finish' and looking after her amazing younger sister Khadija (two year old) so that I could do some writing. And last, but certainly not least, my mother - Dr Naheed Sultana - for her unconditional love, inspiring me to work hard and excel and telling me off when I delayed writing up this thesis.

1. Chapter 1 – Introduction

1.1 The Pancreas

1.1.1 Anatomy (acinar unit, ductal system, acinar cell)

The word pancreas is derived from the Greek word *pan-kreas* meaning *'*all flesh', with regards to its fleshy appearance. The discovery of pancreas has been attributed to the Greek anatomist, Herophilos (335-280 BC), who performed the first systematic dissections of human cadavers (Busnardo, DiDio et al. 1983). The pancreas, embryologically derived from the foregut (endoderm), is a glandular organ located in the posterior aspect of the upper abdominal cavity in humans. It is anatomically divided into three parts; the head, body and tail (figure 1.1). The head of the pancreas abuts the duodenum and the tail extends towards the spleen. It has both endocrine and exocrine functions. The functional enzyme-secreting unit of the exocrine pancreas is the acinus, a Latin name relating to its 'grape like' appearance (Bockman, Boydston et al. 1983). The acini are formed by the closely packed, terminal secretory units of zymogen secreting acinar cells grouped around a central common lumen, as well as a few centro-acinar cells located around the beginning of the ductal system. The intercalated ducts, which secrete HCO_3^- and water in response to secretin, along with the acinus form the acinar ductal units (figure 1.1). The enzymes produced by the pancreas include; trypsinogen, pepsinogen, amylase, lipase and deoxyribonuclease which are secreted as inactive pro-enzymes and activated in the duodenum by enterokinase to prevent pancreatic autodigestion.

The pancreatic acinar cell (PAC) is the classical, polarised, secretory cell, with intracellular Ca^{2+} playing a key role in the control of its activities (Petersen and Sutton 2006). These non-excitable exocrine cells have a high

secretory turnover heavily dependent on mitochondrial production of adenosine triphosphate (ATP) (Petersen and Tepikin 2008). The organellar arrangement of the PAC is such that the endoplasmic reticulum (ER) is densely packed around the nucleus in the basolateral region extending into the apical region, where the Golgi extends into the zymogen granules (ZG) (Tinel, Cancela et al. 1999). Mitochondria surround the apical pole, nucleus and the basolateral plasma membrane, providing the energy required for the essential functions of these regions (Park, Ashby et al. 2001).

The endocrine function of the pancreas is regulated by clusters of cells referred to as Islet of Langerhans, which comprise approximately 2% of the pancreatic tissue. There are approximately three million pancreatic islets in the human pancreas (Ionescu-Tirgoviste, Gagniuc et al. 2015), primarily responsible for glucose metabolism. There are four different types of cells in the pancreas islets, each secreting a different hormone. α alpha cells secrete glucagon (increase glucose in blood), β beta cells secrete insulin (decrease glucose in blood), δ delta cells secrete somatostatin (regulates/stops α and β cells) and PP cells, or γ (gamma) cells, secrete pancreatic polypeptide (Constanzo 2006). Histologically, the capillaries of the islets are lined by layers of islet cells and most endocrine cells are in direct contact with the blood vessels. The function of islets is independent from the digestive role of the majority of PACs.

Since the exocrine pancreas forms the bulk of the gland and is the primary site of insult in acute pancreatitis (AP), it will be the main focus of this thesis.

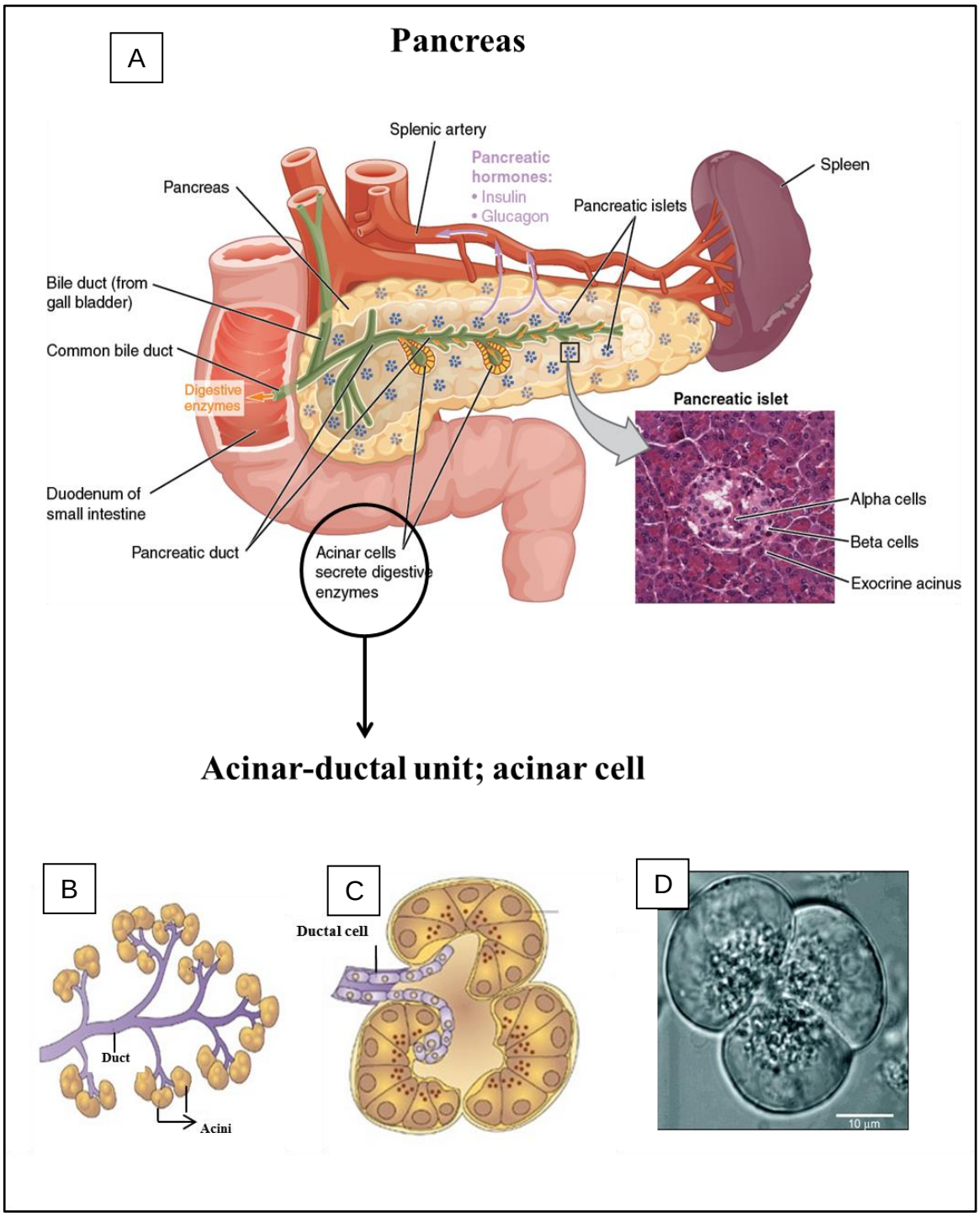


Figure 1.1 - Anatomy of the Pancreas

Gross anatomy of the pancreas (A). Acinar ductal unit (B) and structure of pancreatic acini, composed of acinar cells grouped around a central lumen (C). Transmitted light image of an isolated acinar triplet (D). *Modified from Gray's Anatomy for students, 2012.*

1.1.2 Physiological signaling and secretory function

Intracellular Ca^{2+} signals control normal secretion in PACs. During physiological stimulation, binding of the hormone CCK or neurotransmitter acetylcholine (ACh) to their respective G-protein linked receptors activates ADP ribose cyclase and phospholipase C to form the second messengers; nicotinic acid adenine dinucleotide phosphate (NAADP), cyclic ADP ribose (cADPr) and inositol trisphosphate (IP3). These second messengers in turn rapidly diffuse and bind to their receptors (IP3R, ryanodine receptors, RyR, and NAADPR), which are Ca^{2+} channels on the ER, ZGs and endolysosomes (ELs) (Ashby and Tepikin 2002, Petersen and Sutton 2006). Consequently, repetitive local rises (spikes) in the free cytosolic Ca^{2+} concentration are produced which are buffered by the peri-apical mitochondria that respond by increasing ATP production to drive secretion, and terminate the signal (Tinel, Cancela et al. 1999, Park, Ashby et al. 2001, Voronina, Sukhomlin et al. 2002) . Ca^{2+}- induced Ca^{2+} release (CICR), notably via RyR prevents the spread of Ca^{2+} signals beyond the peri-apical buffer zone into the basolateral region of the cell, again driving mitochondrial ATP production for transcription, translation and ATPase pump activity (Ashby and Tepikin 2002). The sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump refills the ER Ca^{2+} store while the plasma membrane Ca^{2+} - ATPase (PMCA) pump assists to restore the normal basal Ca^{2+} (Burdakov, Petersen et al. 2005) and these two pumps also clear a continuous ER Ca^{2+} leak. Ca^{2+} entry into the cell is regulated at the plasma membrane by store-operated Ca^{2+} channels (Parekh and Putney 2005). Since PAC is a non excitatory cell, extracellular Ca^{2+} entry does not typically initiate signaling, however sustains continued signaling, and is required to replace PMCA-

mediated extrusion and after secretion, to replenish stores that will contribute to new ZGs (Petersen 2005). Since the discovery of the Ca^{2+} entry channel ORAI1, it has been shown to be the principal store operated calcium entry (SOCE) channel in the PAC (Lur, Haynes et al. 2009). Opening of the ORAI1 channel is in turn regulated by the stromal interaction molecules (STIM1 and STIM2), following decreases in ER Ca^{2+} store concentrations (Lur, Haynes et al. 2009, Muik, Schindl et al. 2012, Derler, Schindl et al. 2013, Gerasimenko, Gryshchenko et al. 2013).

1.2 Clinical acute pancreatitis

Acute pancreatitis is a disease characterized by acute inflammation of the pancreas (Pandol, Saluja et al. 2007). It was first described in 1889 by Reginald Fitz, a pathologist at Harvard, when he reported the pathologic findings distinguishing; haemorrhagic, suppurative, and gangrenous forms of the disease (FITZ 1889) and (Busnardo, DiDio et al. 1983). Acute pancreatitis has also been described as the 'most terrible of all calamities that occur in connection with the abdominal viscera'(Moynihan 1925).

1.2.1 Aetiology

Gallstones and excessive alcohol intake are the two most common causes of AP, accounting for 40% and 30% of the cases respectively (Forsmark, Vege et al. 2016). The prevalence of gallstone disease can exceed 20% in the adult population according to population-based studies (Volzke, Baumeister et al. 2005). It has been established that gallstones within the gallbladder do not cause pancreatitis and that the initiation of pancreatitis requires the passage of a gallstone from the gallbladder through the biliary tract (Acosta and Ledesma 1974). There are two hypotheses relating to gallstone-induced pancreatitis pathogenesis, both proposed by Eugene Opie in 1901(Pandol, Saluja et al. 2007). According to the 'duct obstruction-impaired secretion hypothesis', a gallstone passing through the biliary tract obstructs the pancreatic duct and the subsequent flow from the exocrine pancreas triggers acinar or duct cell damage . Opie's 'common channel - bile reflux hypothesis' on the other hand

postulates that a gallstone impacted at the duodenal papilla creates a communication between the pancreatic and common bile ducts. This allows flow of bile through this “common channel” into the pancreatic duct and triggers pancreatitis. It is now understood that migrating gallstones cause transient obstruction of the pancreatic duct, a mechanism shared by other recognized causes of AP such as; endoscopic retrograde cholangiopancreatography (ERCP), pancreas divisum and sphincter of Oddi dysfunction (Forsmark, Vege et al. 2016).

The overall lifetime risk of pancreatitis among heavy drinkers is 2 to 5% where prolonged alcohol use is characterised by four to five drinks daily over a period of more than five years (Cote, Yadav et al. 2011). The risk is higher for men than for women, perhaps reflecting differences in alcohol intake or genetic background (Whitcomb, LaRusch et al. 2012). The type of alcohol ingested does not affect risk, and objective analysis during the Oktoberfest in Germany has revealed that binge drinking in the absence of long-term, heavy alcohol use does not precipitate AP (Phillip, Huber et al. 2011).

The World Health Organization database includes 525 different drugs suspected to cause pancreatitis and drug induced AP accounts for less than 5% of all cases of pancreatitis. The most strongly associated drugs with AP are; azathioprine, 6-mercaptopurine, didanosine, valproic acid, angiotensin-converting-enzyme inhibitors and mesalamine (Nitsche, Maertin et al. 2012). Population based studies have also shown a dose-dependent association between increasing polypharmacy and risk of AP (Razavi, Lindblad et al. 2016).

A number of genetic mutations and polymorphisms have been associated with acute (and chronic) pancreatitis. These include mutations in the genes encoding; cationic trypsinogen (PRSS1), serine protease inhibitor Kazal type 1 (SPINK1), cystic fibrosis transmembrane conductance regulator (CFTR), chymotrypsin C, calcium-sensing receptor and claudin-2 (Whitcomb 2013).

Hyperlipidaemia is a rare cause of AP in the Western population; however it is ranked second as the aetiology of pancreatitis in China, accounting for up to 14.3% of the cases (Zhu, Pan et al. 2017). Other causes of AP include; autoimmune pancreatitis, ERCP (5-10%), trauma, surgical complications of coronary artery bypass grafting (5-10%), malignancy and infections secondary to viruses (CMV, EBV and mumps being the most common) or parasites (ascaris and clonorchis) (Forsmark, Vege et al. 2016). Other conditions that have been associated with AP include obesity, diabetes and smoking (Hong, Qiwen et al. 2011, Yadav and Lowenfels 2013).

1.2.2 Epidemiology

The epidemiology of AP has been objectively evaluated by a designated taskforce established by United European Gastroenterology, who reported that the incidence of AP ranges from 4.6 to 100 per 100 000 population (Roberts, Morrison-Rees et al. 2017). The incidence is highest in eastern and northern Europe with a consistent trend showing an increase in incidence over time. Analysis of aetiology amongst different geographical regions revealed cholelithiasis as the commonest aetiology in southern Europe, alcohol in eastern Europe and intermediate ratios in northern and western Europe

(Roberts, Morrison-Rees et al. 2017). In the United States, AP accounts for 275,000 hospital admissions each year, with a rise in admissions by 20% over the last 10 years, costing the healthcare service approximately $2.5 billion per annum (Peery, Crockett et al. 2015). In the UK, AP accounts for approximately 26,000 hospital admissions per year (van Dijk, Hallensleben et al. 2017).

The incidence and outcome of pancreatitis can be influenced by social status. Ellis et al, in an epidemiological study across all patients admitted with AP in North East England showed that compared to the affluent, individuals from the most deprived sections of society were almost 2.5 times more likely to develop AP. People belonging to low socio-economic status were also 6.5 times more likely to have alcohol related disease. The increase in incidence was not only confined to alcoholic AP but also in biliary AP, to a lesser degree (Ellis, French et al. 2009).

1.2.3 Clinical course

Acute pancreatitis is diagnosed in the presence of at least two of the following three diagnostic features (Banks, Bollen et al. 2013):

(i) upper abdominal pain consistent with AP,

(ii) serum lipase or amylase levels that are at least 3 times the upper limit of the normal range,

(iii) findings consistent with features of AP on cross-sectional imaging (computed tomography or magnetic resonance imaging).

The majority of patients with AP, up to 80%, have a mild, self-limited episode. Approximately 20% of the patients have a severe episode defined by the

presence of complications that are systemic, local, or both. Systemic complications of AP include failure of an organ system or exacerbation of a pre-existing disorder and early organ failure is responsible for the majority of deaths in AP (figure 1.2). Local complications include fluid collections around the pancreas and pancreatic necrosis, which can be sterile or infected (Banks, Bollen et al. 2013). Infected pancreatic necrosis accounts for the later organ failure in patients with severe AP (figure 1.3). Although the mortality associated with AP has decreased over time, overall mortality is now approximately 2%, it can approach up to 30% in patients with persistent organ failure (i.e lasting more than 48 hours) (Yadav and Lowenfels 2013). Pancreatic necrosis is a key determinant of outcome in AP, with mortality rates of up to 43% in patients who have infected pancreatic necrosis and organ failure (Petrov, Shanbhag et al. 2010). Other predictors of mortality include old age, multiple co-morbidities (Hong, Qiwen et al. 2011, Krishna, Hinton et al. 2015) and hospital acquired infections (Wu, Johannes et al. 2008).

Despite numerous concepts, attempts and randomised controlled trials (RCTs) there is still no specific treatment for AP. In fact, despite the unmet clinical need for specific therapy for AP, a recent analysis of research trends in Gastroenterology over the last 50 years has identified a substantial drop in the research activity on AP (Szentesi, Toth et al. 2016). The significant improvement in the prognosis of patients has been due to the improved intensive care and less-invasive interventional techniques. There has however been an increase in the incidence of iatrogenic AP due to the increase in number of interventional ERCP's. The most important development over the recent years has been the conceptual change in the management of

necrotizing pancreatitis from a primarily surgical disease to a non-surgical management, electively supported by late minimal interventions(van Grinsven, van Santvoort et al. 2016, van Brunschot, Hollemans et al. 2017). There are further opportunities to improve the outcome of patients with pancreatitis, such as rationalising the use of antibiotics in AP (Baltatzis, Jegatheeswaran et al. 2016) and management of gallstones in patients with gallstone pancreatitis (Siriwardena and O'Reilly 2017). Findings of the 2016 UK National Confidential Enquiry into Patient Outcome and Death (NCEPOD) evaluating the care provided to patients admitted to hospital with AP identified that only 56% of hospitals treating patients with AP reported that patients with gallstone pancreatitis underwent cholecystectomy either during the index admission or within 2 weeks (Siriwardena and O'Reilly 2017) as recommended by the guidelines (Working Group 2013). Results of the Dutch multicentre randomized PONCHO trial, comparing cholecystectomy during index admission with interval surgery in patients with mild gallstone AP, provides good evidence that early surgery reduces the rate of recurrent gallstone-related complications without an increase in surgical morbidity (da Costa, Dijksman et al. 2016).

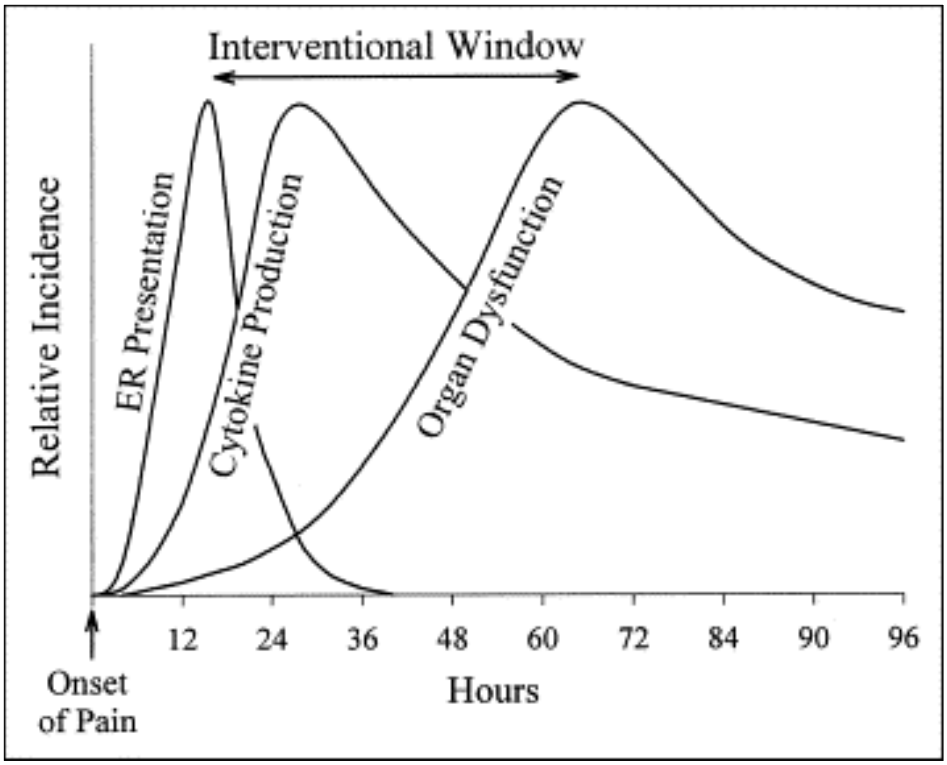


Figure 1.2 - Therapeutic window for treatment of AP

Time course of AP progression, cytokine profile and onset of organ dysfunction. Majority of patients develop systemic complications 2-4 days after onset of symptoms, providing a window of opportunity for intervention. *Adapted from (Norman 1998).*

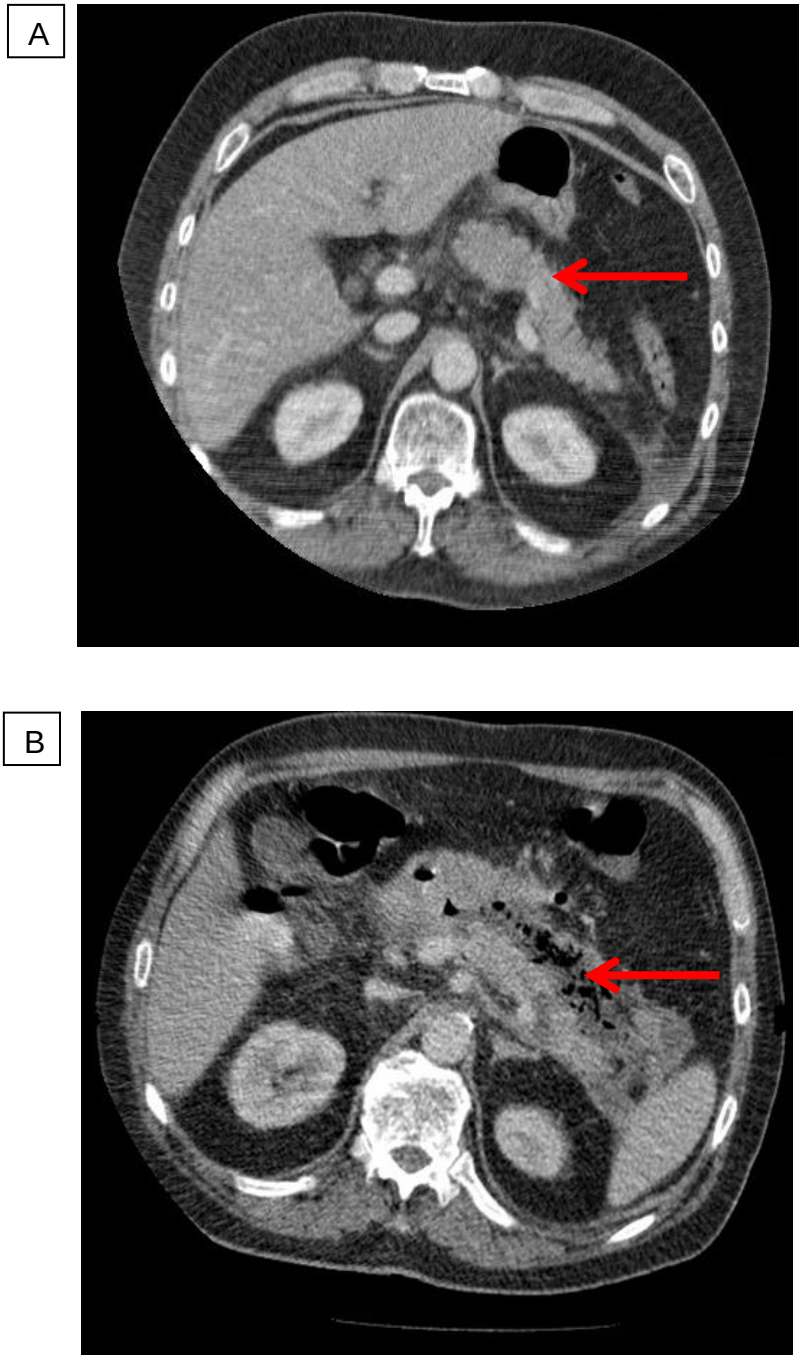


Figure 1.3 - Pancreatic necrosis

Contrast-enhanced axial computed tomography images showing normal pancreas (A). In cases of AP complicated by infected pancreatic necrosis, the pancreas gland is swollen along with areas of hypoperfusion compatible with pancreatic necrosis and presence of gas indicates infected pancreatic necrosis (B).

1.3 Pathophysiology of acute pancreatitis

1.3.1 Pathological calcium signaling

Disruption of physiological calcium signaling, resulting in sustained cytosolic calcium overload within the PAC, is a crucial step in the pathobiology of AP (Ward, Sutton et al. 1996, Kruger, Albrecht et al. 2000, Raraty, Ward et al. 2000). Pancreatic toxins including bile acids (Voronina, Gryshchenko et al. 2005), oxidative (Shalbueva, Mareninova et al. 2013) and non-oxidative metabolites (Criddle, Murphy et al. 2006, Huang, Booth et al. 2014) of ethanol and cholecystokinin (CCK) hyperstimulation (Murphy, Criddle et al. 2008, Criddle, Booth et al. 2009). These toxins lead to abnormal elevations of Ca^{2+} that are global and sustained which induce premature intracellular enzyme activation, mitochondrial dysfunction, impaired autophagy, vacuolization, and activation of necrotic cell death pathway; all of which contribute towards the pathophysiology of AP (Criddle, McLaughlin et al. 2007). In order to maintain this sustained cytosolic calcium overload, continuous emptying of the ER Ca^{2+} store and activation of SOCE and Ca^{2+}-release activated Ca^{2+} currents (CRAC) is required in order to replenish the ER store (Gerasimenko, Gryshchenko et al. 2013, Shalbueva, Mareninova et al. 2013).These findings are supported by experimental data demonstrating that Ca^{2+} chelation, which attenuates cytosolic calcium overload, prevents activation of ZGs and vacuolization in PACs (Saluja, Bhagat et al. 1999, Raraty, Ward et al. 2000) and also reduces severity of EAP (Mooren, Hlouschek et al. 2003). The inositol 1,4,5-trisphosphate receptor (IP3R) Ca^{2+} channels are primarily responsible for the release of Ca^{2+} from intracellular stores (Gerasimenko, Lur et al. 2009). This

IP3R mediated sustained elevation in cytosolic calcium can be inhibited by genetic deletion in the form of double knockout IP3R types 2 and 3 mice (Futatsugi, Nakamura et al. 2005) or pharmacological inhibition with caffeine (Gerasimenko, Lur et al. 2009).

1.3.2 Trypsinogen activation in acute pancreatitis

The Austrian pathologist Hans Chiari in 1896 was the first to propose that pancreatitis was a disease of autodigestion in which 'the organ succumbs to its own digestive properties' (pancreadpedia 2017). Trypsin is synthesised in the PAC in its inactive form – trypsinogen within the rough ER and subsequently transported to the Golgi apparatus for sorting. Trypsinogen is always co-synthesised and packed alongside a pancreatic secretory trypsin inhibitor (PSTI) which inhibits its premature activation. Within the Golgi apparatus, trypsinogen and other digestive enzymes are condensed in the form of ZGs. In this condensed state the enzymes are stable and not susceptible to activation. In response to secretory stimuli, these ZGs are released in to the lumen of the pancreatic duct, and on reaching the duodenum activated by the action of enteropeptidases, which remove the 7-10 amino acid chain from the N-terminal region of trypsinogen known as trypsinogen activation peptide (TAP). Removal of TAP induces a conformational change that results in active trypsin (Abita, Delaage et al. 1969).

Under pathological conditions, lysosomal enzymes and zymogens fuse to form 'co-localisation organelles' and the lysosomal protease cathepsin B activates trypsinogen leading to the activation of trypsin within the pancreas

(Saluja, Hashimoto et al. 1987, Teich, Bodeker et al. 2002). In EAP, trypsin inhibition with the use of protease inhibitors has shown to ameliorate the severity of disease (Van Acker, Saluja et al. 2002, Van Acker, Weiss et al. 2007) and expression of active trypsin in the pancreas can induce AP (Gaiser, Daniluk et al. 2011). Furthermore genetic deletion or pharmacological inhibition of cathepsin B, both of which limit trypsinogen activation, reduce pancreatic injury in AP (Halangk, Lerch et al. 2000). It is however interesting to note that inhibition of trypsinogen activation reduces local pancreatic injury but does not have any significant impact on leucocyte infiltration in the pancreas or lung (Halangk, Lerch et al. 2000). Further studies using the genetically modified mouse lacking trypsinogen isoform-7 also revealed that trypsin is only partly responsible for PAC necrosis in AP, and local and systemic inflammation is independent of trypsin (Dawra, Sah et al. 2011). Trypsin induces PAC injury by making the co-localised vesicles fragile, allowing cathepsin B to escape into the cytosol and subsequent activation of apoptotic cell death pathway (Talukdar, Sareen et al. 2016). Interestingly, the amount of cathepsin B in the cytosol also determines the mode of cell death in PACs. Small quantities of cathepsin B activate apoptosis, whereas larger amounts lead to activation of necrotic cell death pathway (Moriwaki and Chan 2013, Talukdar, Sareen et al. 2016).

1.3.3 Cytokines and inflammation in acute pancreatitis

Pancreatic acinar cells synthesise and release cytokines and chemokines in response to injury, (Grady, Liang et al. 1997, Gukovskaya, Gukovsky et al. 1997, Norman 1998) and upregulate the expression of adhesion molecules

such as the intercellular adhesion molecule-1 (Zaninovic, Gukovskaya et al. 2000). Cell death also leads to the release of damage-associated molecular patterns (DAMPs); such as histones, high-mobility group box1 protein (HMGB1), nuclear and mitochondrial DNA, heat shock proteins and ATP (Kang, Lotze et al. 2014, Yu, Wan et al. 2014) which initiate an acute inflammatory response. These factors promote neutrophil and monocyte infiltration (Frossard, Saluja et al. 1999) and can exacerbate tissue injury (Bhatia, Saluja et al. 1998, Frossard, Saluja et al. 1999, Gukovsky, Li et al. 2013). There is a complex interplay of several pathways, mediators and inflammatory cells that leads to the inflammatory response in AP that can contribute to early onset organ failure in patients with AP (Oiva, Mustonen et al. 2010, Oiva, Mustonen et al. 2013). The recruitment and activation of neutrophils and macrophages contributes to elevated levels of pro-inflammatory mediators such as tumor necrosis factor (TNFα), interleukins (IL) – IL-1, IL-2, IL-6, and other chemokines and anti-inflammatory factors such as IL-10 (Davies and Hagen 1997, Makhija and Kingsnorth 2002). TNFα, a soluble inflammatory cell mediator, is a product of the activated NF-κB pathway. It is also shown to directly induce premature trypsinogen activation and necrosis in PACs, suggesting a contribution of inflammatory signaling in disease initiation and progression (Sendler, Dummer et al. 2013).

The magnitude of cellular injury and the extent of inflammatory response determine the severity of disease. Although in most cases of human AP, cellular injury and local inflammation associated with pancreatitis resolve spontaneously, in some cases, the disease progresses to systemic illness. Systemic inflammatory response syndrome (SIRS) is a result of uncontrolled

local inflammation and predisposes to acute respiratory distress syndrome (ARDS) and multiple organ failure (Robertson, Basran et al. 1988). A summary of the important cytokines and chemokines mediating the inflammatory response in AP is presented in table 1.

The concept of compensatory anti-inflammatory response syndrome (CARS) explains how prolonged disease activity is associated with immune anergy in AP. CARS is primarily mediated by TGF-β, IL-4, IL-10, and CCL2 which are released by neutrophils and monocytes (Takahashi, Tsuda et al. 2006, Ho, Chiu et al. 2011). These cytokines are responsible for promoting a Th2-type adaptive immune response, thereby increasing susceptibility to superinfections (Kobayashi, Kobayashi et al. 1998). Evaluation of cytokine profile of patients in human AP reveals that the temporal relation and magnitude of pro- and anti-inflammatory cytokine release is similar in patients, (Gunjaca, Zunic et al. 2012) with peak cytokine concentration within 48 hours of disease onset. These findings indicate that the anti-inflammatory cytokines play an important role in limiting the extent of SIRS.

Table 1 - Key cytokines and chemokines mediating the inflammatory response of pancreatitis

Signaling molecule	***Source in AP***	***Receptors and targets***	***Function***
Cytokines			
TNFα	Acinar cells, endothelium, monocytes, Kupffer cells	TNFR1: widely expressed; TNFR2: immune and endothelial cells	Pro-inflammatory; regulates apoptosis, mediates trypsin activation in acinar cells
IL-1β	Pancreas (beta cells, stellate cells), lung, liver, spleen, monocytes	Secreted as a pro-enzyme, converted by ICE or neutrophil proteases to its active form; acts on IL-1R (widely expressed)	Pro-inflammatory; increases vascular permeability. Soluble IL-1ra inhibits IL-1β activity
IL-6	Ubiquitous expression	IL-6R: hepatocytes, neutrophils, macrophages; soluble sIL-6R mediates trans-signaling	Pro-inflammatory; contributes to lung injury in AP; lethal if administered in the context of EAP
IL-10	Lymphocytes (B- and T-), monocytes/macrophages, dendritic cells, Kupffer cells	IL-10R: widely expressed	Anti-inflammatory; inhibits pro-inflammatory cytokine release from lymphocytes via STAT3; downregulates MHCII costimulatory molecules CD80/CD86, reducing clonal expansion of T-lymphocytes
Chemokines			
CCL2	Acinar cells and possibly other cell types in the pancreas	CCR2, CCR4	Pro-inflammatory, monocyte chemo-attractant, mediates pancreas and lung injury in EAP
CXCL1/2	Acinar cells, macrophages	CXCR2: neutrophils and myeloid derived suppressor cells	Pro-inflammatory, strong neutrophil chemo-attractants

Modified from (Szatmary 2016)

1.3.4 Endoplasmic stress in acute pancreatitis

The endoplasmic reticulum (ER) are key cellular organelles involved in metabolic processes such as; folding and aggregation of proteins, gluconeogenesis, lipid synthesis and intracellular calcium storage (Fu, Watkins et al. 2012). The initial steps of protein maturation that take place at the ER are crucial for the proper folding of proteins that regulate secretory pathways (Hetz 2012). The dynamic changes in the protein-folding requirements are tightly regulated by feedback mechanisms which affect almost every aspect of the secretory pathway (Ron and Walter 2007). The protein-folding machinery in the ER of the PAC is constantly under stress due to the high demand for protein synthesis. The quality control function of ER is activated when proteins are subjected to improper processing or modification. These unfolded or misfolded proteins are retained within the ER and subsequently cleared through the ubiquitin-proteasome pathway (UPP) or ER-associated degradation (ERAD) (Kozutsumi, Segal et al. 1988). Factors such as oxidative stress and alterations in calcium homeostasis can lead to the accumulation of excessive misfolded proteins within the ER, leading to ER stress (ERS) (Wu, Li et al. 2016). Feedback mechanisms are activated in response to ERS, protecting cells against endogenous and exogenous stresses. Through a number of signal transduction pathways, the protein folding and degradation capacity of the cell are augmented whereas translation of most proteins is stopped thereby reducing the burden on the organelle by decreasing the number of proteins in the ER. These effects are achieved by the unfolded protein response (UPR) (Boyce and Yuan 2006). In eukaryotes, the three ER membrane-associated proteins that regulate the UPR in ER include:

(i). protein kinase RNA-like ER kinase (PERK),

(ii). inositol-requiring enzyme 1 (IRE1) and

(iii). activating transcription factor 6 (ATF6).

During physiological conditions, these three proteins bind to the ER chaperone immunoglobulin heavy chain-binding protein (BiP) and maintain signal transduction factors in a non-activated state. Under conditions of ERS, as the unfolded proteins aggregate within the ER lumen, these chaperone proteins dissociate from the luminal surface of the ER membrane proteins and bind to unfolded proteins. This binding activates the other ER membrane proteins and leads to the initiation of UPR (Hotamisligil 2010). The level and duration of ERS dictates the extent to which each UPR pathway becomes activated. Initially, new protein synthesis is attenuated to limit the protein load entering the ER. Sustained ERS leads to induction of gene expression via nuclear signaling to promote protein folding and degradation of misfolded proteins. When ER damage becomes irreversible and normal functions cannot be restored, apoptotic cell death pathway is activated (Hotamisligil 2010).

Pancreatic acinar cells contain abundant ER in order to meet the physiological demands of protein synthesis. The newly synthesised digestive enzymes are transported to the ER and bind to BiP, which exhibits ATPase activity. BiP relies on the energy produced by ATP hydrolysis to accomplish proper folding and post-translational modifications of proteins, which are assembled into ZGs in the Golgi (Low, Shukla et al. 2010). Given the high rates of protein synthesis and energy requirements, PACs are more susceptible to external stimuli in case of ERS (Kubisch and Logsdon 2008). There is evidence

to suggest that ERS plays a vital role in the development and progression of AP (Thrower, Gorelick et al. 2010). Alterations in the morphology of ER and formation of vesicle particles (Bhatia, Neoptolemos et al. 2001) have also been observed in experimental pancreatitis, indicating the role of ERS in the pathogenesis of AP (Hartley, Siva et al. 2010, Deng, Chen et al. 2011). Furthermore, these observations were associated with the activation of ERS-related receptors PERK, IRE1 and ATF6 along with their downstream pathways (Kubisch, Sans et al. 2006). ERS has also been shown to induce inflammatory response mediated via NF-κB-dependent pathway in several diseases (Kitamura 2011, Walter and Ron 2011). In experimental studies, ERS induced NF-κB activation and PAC necrosis leads to the development of pancreatitis (Sah, Garg et al. 2012, Huang, Liu et al. 2013, Sah, Garg et al. 2014). Recent studies have shown that ERS destroys PACs due to the action of misfolded trypsin by gene mutation (Masamune 2014). The activation of UPR pathways has also been demonstrated in several models of EAP, implicating the role of ERS in the pathogenesis of AP (Kubisch, Sans et al. 2006, Kowalik, Johnson et al. 2007, Ye, Mareninova et al. 2010, Fazio, Dimattia et al. 2011, Seyhun, Malo et al. 2011).

1.3.5 Heat shock proteins

Heat shock proteins (HSPs) are highly conserved proteins expressed in response to stress in all species. These were identified in initial experiments where exposure of heat shock lead to the synthesis of certain polypeptides and it was later discovered that these proteins are upregulated in response to other

stresses including; inflammation, ischaemia, anoxia and exposure to heavy metals (Tissieres, Mitchell et al. 1974). These proteins have a protective role against toxic inflammatory mediators (Polla, Perin et al. 1993, Jacquier-Sarlin, Fuller et al. 1994) whose synthesis is mediated via activation of transcription factor heat shock factor-1(HSF-1) (Morimoto 1993), under conditions of stress. Experimental evidence suggests that synthesis of HSP27 and HSP70 is increased in isolated pancreatic acini as well as in the pancreata of animals with caerulein-induced pancreatitis (Weber, Gress et al. 1995, Ethridge, Ehlers et al. 2000, Bhagat, Singh et al. 2002) and other models of EAP (Weber, Wagner et al. 2000, Tashiro, Schafer et al. 2001). Furthermore severity of caerulein-induced AP is much more pronounced in HSF-1 knockout mice whereas overexpression of HSP27 ameliorates severity of AP (Frossard, Pastor et al. 2001). Pre-exposure to sublethal stress, by water immersion or exposure to hyperthermia, which leads to expression of HSPs, has also been found to have a protective effect on severity of EAP (Weber, Gress et al. 1995, Wagner, Weber et al. 1996, Tashiro, Schafer et al. 2001). HSP70 prevents the ZG/lysosomal co-localisation thereby reducing intracellular activation of trypsinogen in PACs (Hietaranta, Singh et al. 2001) and overexpression of HSP 70 also provides protection against NFkB activation (Bhagat, Singh et al. 2008). Clinical studies correlating the severity of AP with HSP70 gene polymorphism status identified that HSP70-2 expression was linked to the severity of pancreatitis. The HSP70-2G allele, which is associated with low HSP70-2 expression, was more prevalent in patients with severe AP compared with mild disease or healthy subjects. On the contrary, patients with the “protective” AA genotype were found to be less vulnerable to severe form of AP (Balog, Gyulai et al. 2005).

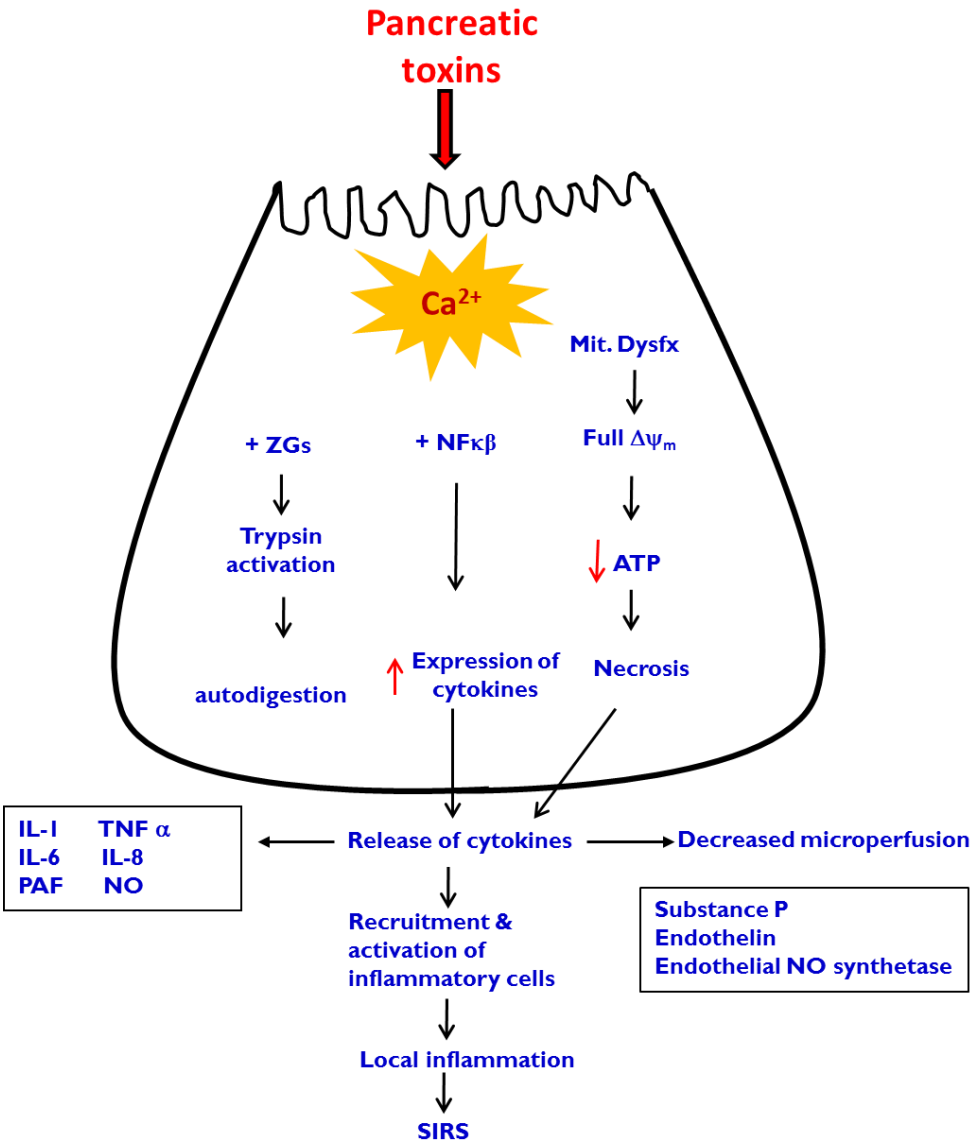


Figure 1.4 - Intracellular and extracellular factors in pathophysiology of AP

This schematic diagram shows that acinar cell injury is the primary event in the pathogenesis of AP and the interplay between extracellular and intracellular factors contributing to the pathogenesis of AP. Cytosolic calcium overload is the hallmark of PAC injury leading to ZG activation, trypsinogen activation, and increased transcription of NFkB and expression of other pro-inflammatory cytokines. Once released, these cytokines contribute to reduced microperfusion and ischaemia as well as recruitment and activation of inflammatory cells which could lead to SIRS. Sustained elevation of cytosolic calcium also leads to ER stress, mitochondrial dysfunction and ATP depletion which drives the cell towards necrosis.

1.4 Pancreatic acinar cell mitochondrial function

1.4.1 Physiological role of mitochondria in pancreatic acinar cell

Mitochondria, within the PAC are classically distributed in the peri-apical, peri-nuclear and sub-plasmalemmal regions (figure 1.5). These organelles are the powerhouses of the PAC, providing energy for various cellular processes including the secretion of inactive digestive enzyme precursors. Mitochondria are unique cellular organelles as they contain their own genome and protein synthesis machinery. Mitochondria respond to physiological calcium signals induced by hormonal and neuronal stimulation by CCK or acetylcholine, respectively (Voronina, Sukhomlin et al. 2002, Murphy, Criddle et al. 2008, Criddle, Booth et al. 2009). This leads to an increase in NADH generation via stimulation of Ca^{2+}-dependent dehydrogenases of the Krebs cycle, feeding into the electron transport chain thereby promoting ATP production. Mitochondria maintain a membrane potential ($\Delta\psi_m$) of 150-180 mV across the inner mitochondrial membrane (IMM), negative inside, by pumping protons into the inter-membrane space (Rizzuto and Pozzan 2006). By regulating the bioenergetics of the cell, mitochondria play an important role in the regulation of cell death pathways in PACs (Criddle, Gerasimenko et al. 2007).

Mitochondria within the acinar cell also play a crucial role in maintaining Ca^{2+} homeostasis (Tinel, Cancela et al. 1999, Park, Ashby et al. 2001, Voronina, Sukhomlin et al. 2002). The peri-apical mitochondria take up local cytosolic Ca^{2+} spikes through the Ca^{2+} uniporter (Kirichok, Krapivinsky et al. 2004) and prevent further spread of the signal which is terminated via the mitochondrial Na+/Ca^{2+} exchanger (Rizzuto and Pozzan 2006). This regulation

of Ca^{2+} signals prevents further emptying of the Ca^{2+} stores, additional large frequent Ca^{2+} fluxes, and reduces metabolic demands for ATP production. In the PAC, physiological Ca^{2+} signals always begin within the apical pole. However under pathological conditions of Ca^{2+} overload, the immediate buffering capacity of peri-apical mitochondria is exceeded, leading to the spread of Ca^{2+} signals into the basolateral region. Ca^{2+} entry into the peri-nuclear and sub-plasmalemmal mitochondria initiates cellular responses in the basolateral region of the cell (Ashby and Tepikin 2002, Petersen 2005). These mitochondrial groups again respond to and assist in termination of the signal, supplying ATP for SERCA and PMCA activity.

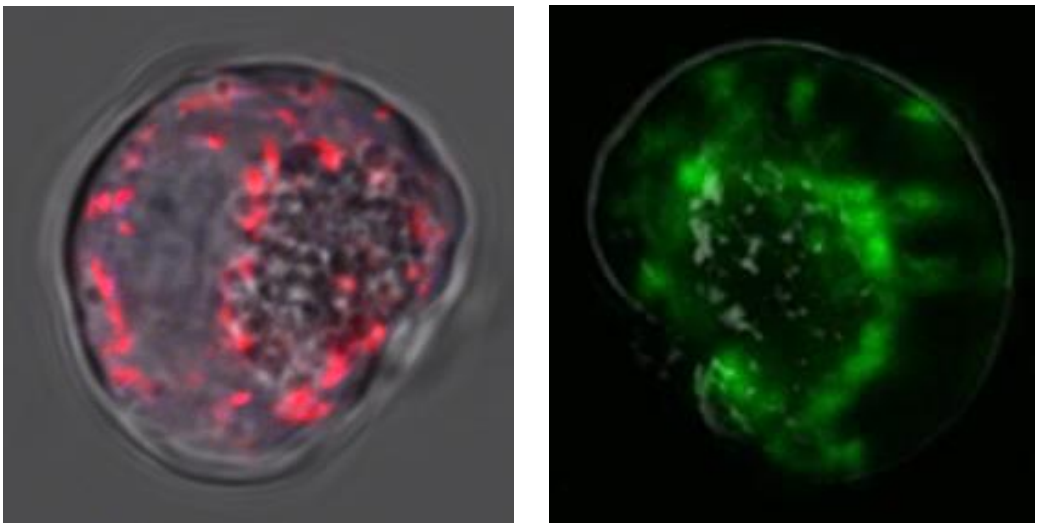


Figure 1.5 - Mitochondrial distribution within PAC

Transmitted light and TMRM (tetramethylrhodamine, methyl ester) shown in red (left) and confocal TMRM flourescence shown in green (right) demonstrating the classical distribution of mitochondria in an isolated murine PAC.

Note: TMRM is a cell-permeant, cationic, fluorescent dye that is readily sequestered by active mitochondria.

Healthy mitochondria are also involved in mitochondrial dynamics, mitophagy, mitochondrial biogenesis and repair (Green and Van Houten 2011). Mitochondrial dynamics refers to a combination of fission and fusion processes (Westermann 2010) which sustain healthy mitochondria as mitochondrial components are mixed during fusion and fission which allows damaged regions of a mitochondrion to be isolated and removed by mitophagy. Mitophagy is triggered by the loss of $\Delta\psi_m$ which activates pathways that lead to the recruitment of autophagic machinery required for the formation of double-membrane vesicles around damaged mitochondria. These vesicles fuse with lysosomes to destroy the mitochondria (Youle and Narendra 2011).

1.4.2 Role of mitochondrial dysfunction and necrosis in acute pancreatitis

The potential role of mitochondrial injury in the pathophysiology of human AP was suggested by the observation of mitochondrial alterations on electron microscopy on patients with AP and gallstones (Lee, Sheen et al. 1988). Furthermore results of phase II trials of the anti-viral agent Fialuridine in the 1990s showed that the drug led to significant mitochondrial injury and patients developed AP amongst other diseases (Honkoop, Scholte et al. 1997). It is now well established that pancreatic toxins cause impairment of mitochondrial ATP production (Orrenius, Zhivotovsky et al. 2003, Criddle, Murphy et al. 2006, Davidson and Duchen 2006, Petersen and Sutton 2006, Rizzuto and Pozzan 2006, Criddle, Gerasimenko et al. 2007, Mukherjee, Mareninova et al. 2015). Non-oxidative metabolism of ethanol and fatty acids (FAs) results in the formation of fatty acid ethyl esters (FAEEs) which have shown to induce

experimental pancreatitis and have been found in high concentrations within pancreatic autopsy samples of people who have died from alcohol intoxication (Lange and Sobel 1983, Werner, Laposata et al. 1997, Werner, Saghir et al. 2002). FAEEs bind to and accumulate within the inner mitochondrial membrane, where hydrolases release locally high concentrations of FAs, which uncouple oxidative phosphorylation, deplete $\Delta\psi_m$ and impair ATP production (Laposata and Lange 1986, Criddle, Murphy et al. 2006). Diminished ATP production reduces the capacity of the acinar cell to clear Ca^{2+}, and hence the peri-granular mitochondria are unable buffer apical Ca^{2+} elevations. The resulting elevation of Ca^{2+} increases the Ca^{2+} load on mitochondria leading to further collapse of $\Delta\psi_m$ and impairment of ATP production (figure 1.6). Cell death via activation of apoptotic cell death pathway is an energy consuming process and this loss of ATP production resulting from mitochondrial dysfunction drives the cell death towards activation of necrotic cell death pathway (Laposata and Lange 1986). Experimental evidence suggests that the aforementioned sequence of events can be prevented by excluding Ca^{2+} from the external medium (Raraty, Ward et al. 2000, Voronina, Longbottom et al. 2002, Criddle, Raraty et al. 2004) or providing supplementary intracellular ATP (Criddle, Murphy et al. 2006).

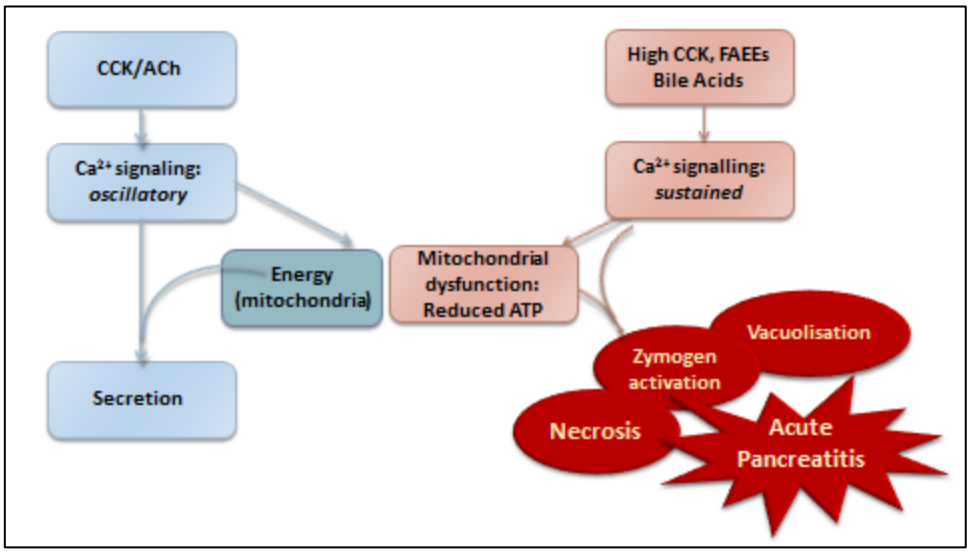


Figure 1.6 - Role of mitochondrial injury in pathophysiology of AP

Physiological calcium signaling is required for secretory function of PAC and is facilitated by ATP production via mitochondria. Pancreatic toxins lead to cytosolic calcium overload, overwhelming the buffering capacity of mitochondria which leads to loss of mitochondrial membrane potential. Mitochondrial impairment inhibits ATP production, activation of mitochondrial protein phosphatase PGAM5 which is a mitochondrial executor of necrosis (Wang, Jiang et al. 2012) resulting in activation of necrotic cell death pathway.

1.4.3 The mitochondrial permeability transition pore (MPTP)

In 1965, Crofts and Chappell reported that energised mitochondria exposed to calcium overload underwent significant swelling characterised by a large decrease in light scattering which could be partially reversed with calcium chelation (Chappell and Crofts 1965). It was initially believed that this swelling was due to a nonspecific permeabilisation of the inner mitochondrial membrane (IMM) via activation of Ca-sensitive lipases (Gunter and Pfeiffer 1990). However, Howarth and Hunter (Hunter and Haworth 1979) and later Crompton et al. (Crompton, Costi et al. 1987) provided evidence that the increased permeability was the sequale of a non-specific channel which became known as the mitochondrial permeability transition pore (MPTP).

The MPTP has now been identified as the instrument of mitochondrial impairment and necrosis in the PAC (Werner, Laposata et al. 1997, Schild, Matthias et al. 1999, Rizzuto and Pozzan 2006, Shalbueva, Mareninova et al. 2013, Mukherjee, Mareninova et al. 2015). Over the last 30 years, a number of models of the MPTP have been proposed. However the opening of the MPTP, now recognised to be formed by the F0F1 ATP synthase (Giorgio, von Stockum et al. 2013, Jonas, Porter et al. 2015), allows an uncontrolled proton flow across the IMM leading to an unregulated entry and exit of ions and solutes up to 1.5 kDa, as well as water, into and out of the mitochondrial matrix (figure 1.7). This results in the loss of inner mitochondrial-membrane potential ($\Delta\psi_m$), essential for ATP production (Halestrap 2010, Rasola and Bernardi 2011). The effect of MPTP opening on mitochondrial dysfunction by Ca^{2+} overload was first demonstrated by addition of Ca^{2+} to isolated cow heart mitochondria (Hunter, Haworth et al. 1976), which resulted in mitochondrial

swelling and rupture of the outer mitochondrial membrane (OMM). MPTP opening is physiological in transitory, low conductance mode releasing calcium and reactive oxygen species (ROS) to match metabolism with workload. However, opening of the MPTP is pathological in persistent, large conductance mode compromising ATP production and inducing cell death by activation of necrotic cell death pathway (Hunter, Haworth et al. 1976, Rasola and Bernardi 2011).

MPTP inhibition using cyclosporin A (CsA) has shown significant cardio-protective effects in the setting of ischaemic/reperfusion injury (Griffiths and Halestrap 1995). Studies from various other biological disciplines have led to a consensus that MPTP formation regulates cell death and hence inhibition of the MPTP affords significant cellular protective capacity (Bernardi, Krauskopf et al. 2006, Baines 2009). It has now been discovered that MPTP opening mediates zymogen activation in AP through impaired autophagy (Mukherjee, Mareninova et al. 2015), which is also considered a key initiating event in the pathobiology of AP (Gukovskaya and Gukovsky 2012). Experimental data suggest that in CER-AP, the levels of microtubule-associated protein 1A/1B-light chain 3 (LC3) -II which is an autophagosomal marker of autophagic activity and p62, another marker for the induction of autophagy and clearance of protein aggregates are significantly reduced in the pancreata of knockout mice in which MPTP formation is inhibited, compared with wild type animals, indicating more efficient autophagy. These findings are consistent with reduced CCK-8-induced trypsin activity in knockout mice compared with wild type animals, despite no differences in the amount of trypsinogen.

The most important regulator of MPTP formation is the mitochondrial matrix protein cyclophilin D (CypD), which has enzymatic peptidyl-prolyl cis-trans isomerase activity, essential for protein folding *in vivo* (Vandenabeele, Galluzzi et al. 2010).

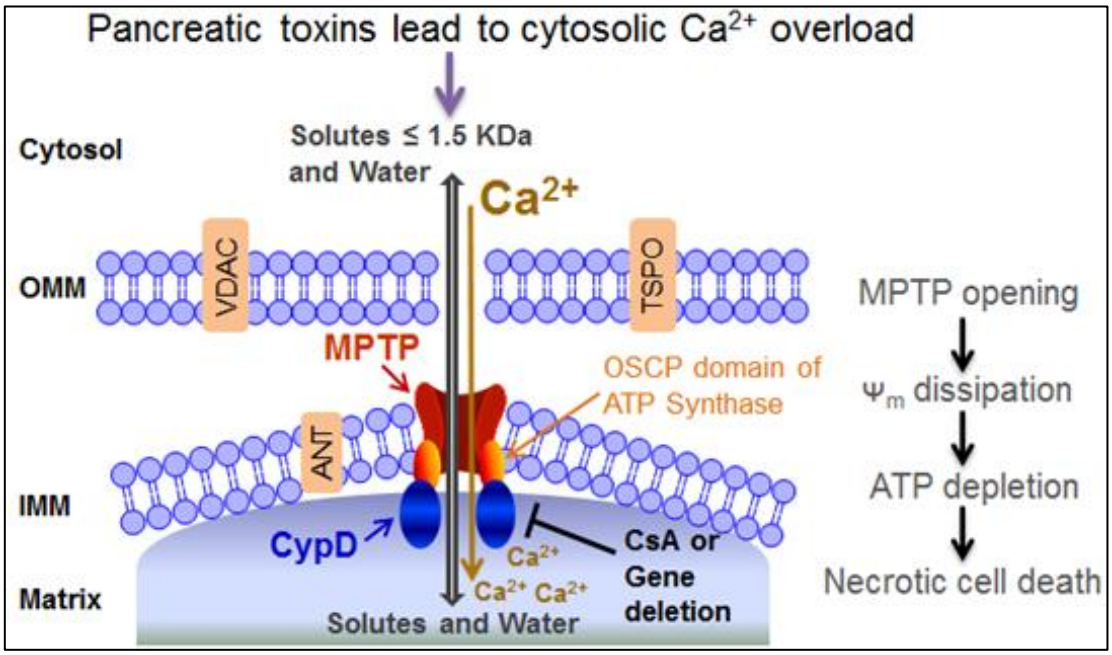


Figure 1.7 - Opening of the MPTP and its consequences

CypD is the most important regulator of the MPTP, which interacts with the oligomycin sensitivity conferring protein (OSCP). The voltage dependent anion channel (VDAC) in the outer mitochondrial membrane (OMM) and adenine nucleotide translocase (ANT) in the inner mitochondrial membrane (IMM) also regulate the MPTP, but are dispensable. The mitochondrial translocator protein (TSPO) also modulates the MPTP but the mechanism is unknown.

1.4.4 Cyclophilin D and cyclophilins

Cylophilins are ubiquitous, highly conserved proteins with sixteen isoforms found in humans, CypA being the most abundant (Wang and Heitman 2005). Cyclophilins were discovered in 1984 by Fischer et al. as these proteins were found to accelerate the cis-trans isomerization of prolyl peptide bonds in short oligopeptides, and subsequently named peptidyl-prolyl cis-trans isomerase (PPIase) (Fischer, Bang et al. 1984). The enzymatic activity of cyclophilins catalyzes a 180° rotation about the C-N linkage of the peptide bond preceding proline and they bind very strongly to CsA (Fischer, Wittmann-Liebold et al. 1989).

CypD is a mitochondrial matrix protein with PPIase activity (Elrod and Molkentin 2013) and recent studies have shown that it binds the lateral stalk of F-ATP synthase (OSCP, b and d subunits) (Giorgio, Bisetto et al. 2009).The definitive proof that CypD is central to necrotic signaling came in 2005 from genetic deletion of the Ppif gene encoding CypD (Baines, Kaiser et al. 2005, Nakagawa, Shimizu et al. 2005, Schinzel, Takeuchi et al. 2005). These studies found that Ppif null cells were highly resistant to cell death induced by cytosolic calcium overload, ROS stress and thapsigargin - an endoplasmic reticulum calcium stressor. The mode of cell death suggested that the role of CypD is prominent in necrotic cell death, but dispensable in apoptotic signaling pathway. Experimental evidence in AP demonstrates that the characteristic local and systemic pathological responses are greatly reduced or abolished in CypD knockout mice confirming that mitochondrial dysfunction through MPTP opening is a fundamental pathological mechanism in AP, which can be

ameliorated by inhibition of CypD (Elrod and Molkentin 2013, Mukherjee, Mareninova et al. 2015).

CsA, a lipophilic cyclic peptide has nanomolar binding affinity for members of cyclophilin family (Handschumacher, Harding et al. 1984), and is an immunosuppressant drug widely used as an anti-rejection drug in solid organ transplantation (Van Buren, Flechner et al. 1984, Borel and Kis 1991). Interaction of CsA with cytosolic CypA generates a complex that has the ability to bind to, and inhibit calcineurin (Wiederrecht, Hung et al. 1992). Consequently, the calcineurin substrate phospho-NFAT is unable to translocate to the nucleus and initiate an immune response (Clipstone and Crabtree 1992, Walsh, Zydowsky et al. 1992). Apart from an immunosuppressant action through CypA, CsA is also a potent inhibitor of MPTP. MPTP inhibition by CsA was demonstrated in two independent studies using isolated rat heart mitochondria (Crompton, Ellinger et al. 1988) and isolated liver mitochondria (Broekemeier, Dempsey et al. 1989), later discovered to occur through binding to mitochondrial CypD (Halestrap and Davidson 1990). These studies provide convincing evidence to target CypD as an appropriate strategy to inhibit the MPTP which is implicated in a variety of other diseases such as, ischaemia-reperfusion injury of the heart, brain and kidney, muscular dystrophies, neuro-degeneration and cancer (Devalaraja-Narashimha, Diener et al. 2009, Wang, Carlsson et al. 2009, He, Wang et al. 2017, Wang, Jiao et al. 2017, Wu, Qian et al. 2017).

The exact role of CypD in physiologically healthy cells remains to be determined. There are some data to suggest that CypD knockout mice display greater levels of anxiety, with reduced tendency to explore and facilitation of

avoidance behaviour. These animals also exhibit an abnormal accumulation of white adipose tissue resulting in adult onset obesity (Luvisetto, Basso et al. 2008). It is however not known whether or not the inhibition of MPTP opening accounts for these effects.

1.4.5 Translocator protein

The translocator protein (TSPO) [18 kDa], which was first described by Braestrup and Squires in 1977 (Gavish, Bachman et al. 1999), is a 169 amino acid protein with five transmembrane domains located primarily on the outer OMM (Anholt, Pedersen et al. 1986). This protein is widely expressed in a number of species and predominantly found in steroid synthesizing tissues including; gonads, kidneys, brain, adrenals and heart (Rupprecht, Papadopoulos et al. 2010). TSPO gene deleted knock-out mice display an embryonic lethal phenotype (Papadopoulos, Amri et al. 1997) indicating the protein's vital role in biological processes.

Synder et al. (Anholt, Pedersen et al. 1986) described that within mitochondria, TSPO can form a multimeric complex with VDAC located on the OMM and ANT located on IMM. TSPO is predominantly concentrated at the level of the intimate contact sites between the two mitochondrial membranes (Culty, Li et al. 1999). This contact site localization of TSPO favours the transport of lipophilic molecules across mitochondrial membranes and is in keeping with its biological function of translocation of cholesterol and porphyrins across the OMM (Taketani, Kohno et al. 1994).

In addition to the role of TSPO in steroid synthesis, it is also involved in other biological functions including apoptosis, cell differentiation and proliferation, mitochondrial porphyrin and protein import as well as regulation of mitochondrial respiration (Morin, Musman et al. 2016). Although the exact structural role of TSPO in the formation of MPTP complex has been controversial (Izzo, Bravo-San Pedro et al. 2016), the TSPO ligand TRO40303

(3,5-Seco-4-nor-cholestan-5-one oxime-3-ol) - initially developed by a French pharmaceutical Trophos, now acquired by Roche - is found to be cardioprotective in MPTP induced ischaemia-reperfusion injury. TRO40303 has shown to reduce infarct size in murine models of ischaemia-reperfusion, provide functional recovery and reduce oxidative stress in the isolated rat heart models (Schaller, Paradis et al. 2010). TSPO may indirectly modulate opening of the MPTP by acting on well-known activators of the pore such as ROS, Ca^{2+} and/or other proteins that have been involved in MPTP opening such as ATP synthase (Cleary, Johnson et al. 2007, Giorgio, von Stockum et al. 2013) and can therefore represent an alternative strategy to develop new pharmacological agents to inhibit MPTP formation. Other potential clinical applications of TSPO include the treatment of various neurological disorders and chemotherapy as certain TSPO ligands have been shown to exhibit anti-proliferative effects against a variety of cancer cell types (Morin, Musman et al. 2016).

1.5 Study aims and objectives

Since MPTP formation plays a critical role in the pathobiology of AP (Lerch, Halangk et al. 2013, Shalbueva, Mareninova et al. 2013, Mukherjee, Mareninova et al. 2015, Ray 2015, Maleth and Hegyi 2016) the overall aim of this translational research project is to evaluate MPTP inhibitors in different stages of drug development as potential therapy of AP. In the first instance a thorough review of the published literature evaluating the current evidence for the treatment of EAP and the translation of these preclinical studies into RCTs in patients with AP was undertaken to identify the pitfalls in pre-clinical studies and understand the reasons for attrition from bench to bedside.

The next part of the study examined the effects of CypD inhibition on mitochondrial protection in response to fatty acid ethyl esters (FAEEs) and the efficacy of CsA and DEB025 (a non-immunosuppressive CypD inhibitor) in reducing necrotic cell death in human PACs. Mitochondrial function is preserved in CypD knockout mice in response to TLCS, however the effect of FAEEs on mitochondrial function in Ppif -/- had not been evaluated. Similarly the protective effects of CsA and DEB025 on necrotic cell death pathway activation had been established in murine PACs but not been studied in human PACs. The first key hypothesis addressed by the initial phase of the study was twofold:

1. ***Genetic deletion of CypD will have similar mitochondrial protection in response to FAEEs when compared with TLCS. Isolated cells from human pancreatic tissue when treated with***

CsA and DEB025 will have similar profile of protection from bile acid induced necrotic cell death as in murine PACs.

Although CsA is a potent inhibitor of the MPTP through CypD binding, the immunosuppressive action of CsA is undesirable in AP, since infection increases mortality in AP. Non-immunosuppressive inhibitors of CypD such as NIM811(Argaud, Gateau-Roesch et al. 2005), DEB025 (Gomez, Thibault et al. 2007) or Sanglifehrin A (Clarke, McStay et al. 2002) have been developed. Like CsA, however, these drugs inhibit all cyclophilins to varying degrees, with inhibitory effects on cell stress responses, e.g. on CypB that facilitates the unfolded protein response in the endoplasmic reticulum. Moreover, poor solubility and large molecular weight also limit the use of these drugs. Therefore, a small molecule inhibitor of CypD with high selectivity would be an ideal target to maintain mitochondrial function by preventing the opening of MPTP in AP. In the next part of this study, a number of novel small molecular inhibitors of CypD were evaluated. Thus, the second key hypothesis addressed in this phase of the study was:

2. ***Inhibition of MPTP using novel small molecular CypD inhibitors will preserve PAC mitochondrial function and reduce necrotic cell death responses following exposure to pancreatic toxins.***

In the last phase of this study an alternative approach was adopted to inhibit MPTP opening in AP. TRO40303 (3, 5-Seco-4-nor-cholestan-5-one oxime-3-ol) a cholesterol-oxime is an MPTP modulator that mediates its affect

by binding to the outer mitochondrial translocator protein (TSPO) and has shown to reduce ischaemia reperfusion-injury (Schaller, Paradis et al. 2010). The efficacy of TRO40303, both *in vitro* and *in vivo*, has been comprehensively studied in this part of the study. Thus, the third key hypothesis in this study was:

3. ***Preservation of mitochondrial acinar cell mitochondrial function using TRO40303 will preserve mitochondrial function and reduce necrotic cell death responses following exposure to pancreatic toxins in vitro, and ameliorate the severity of experimental acute pancreatitis in vivo.***

2. Chapter 2 – Methods

2.1 Solutions

Na HEPES buffered salt solution

The standard isolation solution used for *in vitro* PAC experiments was Na HEPES buffered salt solution, contained the following chemicals (in mM) dissolved in distilled water : NaCl 140, KCl 4.7, $MgCl_2$ 1.13, HEPES 10, glucose 10, $CaCl_2$ 1. The pH was adjusted to 7.3 using NaOH and osmolarity checked at 300±10 mOsm. Agents used during experimentation were diluted in this solution from stock solutions.

2.2 Animals

Wild type CD1 mice were purchased from Charles River laboratories. Typically, male CD1 mice about 8-12 weeks old, weighing about 30 grams were used for PAC isolation and *in vitro* experiments. C57Bl/6 male mice over 25 grams each from Charles Liver laboratories were used for *in vivo* experiments. CypD deficient mice (Ppif-/-) generated by the targeted deletion of Ppif gene encoding for mitochondrial CypD (Baines, Kaiser et al. 2005) were purchased from Jackson laboratory (Jax mice – USA). The first three coding exons were replaced with a neomycin resistance cassette. A 129-dervived embryonic stem cell line was used to create the mutation. Chimeras were crossed to C57BL/6 and the strain was maintained on a mixed C57BL/6 and 129 genetic background by the donating laboratory. Subsequently breeding pairs were used to develop a colony which was maintained in Liverpool. Genotyping was performed to confirm absence of CypD protein (section 2.5). All mice were

housed in standardized conditions with a 12-hour dark/light cycle with free access to water and standard rodent chow. All animal procedures were performed in accordance with protocols defined by the Scientific (Animal) Procedures Act 1986.

2.3 Preparation of isolated pancreatic acinar cells

2.3.1 Isolation of murine pancreatic acinar cells

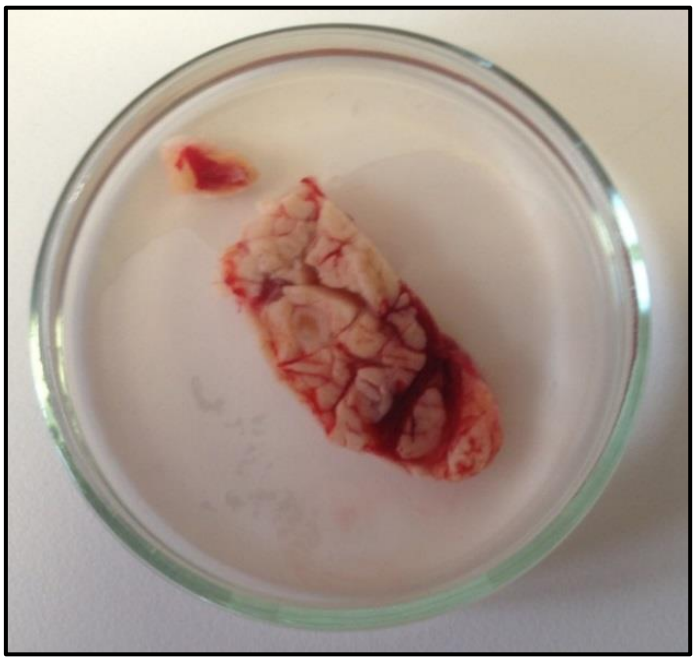
Mice were sacrificed by cervical dislocation in accordance with the Schedule 1 procedures of Home Office. The peritoneal cavity was exposed via midline incision and pancreas identified at the point of attachment to the spleen. The pancreas was dissected, immediately placed in 4-5 ml of Na HEPES buffered salt solution and then injected with 1ml of warm collagenase (220 units/ml, Worthington Biochemical Corporation, Lakewood, NJ) solution at multiple points to introduce collagenase into the ductal structure. The pancreas was incubated at 36.5 °C for 18 to 20 minutes. After collagenase incubation the pancreas was placed in a 15 ml polycarbonate tube (Sarstedt, Leicester UK) with 4 ml of Na HEPES solution. The pancreatic tissue was dissociated mechanically using micropipette tips of progressively decreasing diameter. A cloudy supernatant was formed by cells suspended in the solution which was filtered through a 70 µm nylon mesh cell strainer (BD Falcon, MA, USA) to remove large clusters of cells and connective tissue to be collected in an additional identical tube and replaced with fresh Na HEPES solution. This process was continued until cloudy solution was no longer obtained. The second tube was then centrifuged at 260 G for 1 minute to form a pellet. This pellet was then re-suspended in Na HEPES solution for loading with fluorescent indicators.

2.3.2 Isolation of human pancreatic acinar cells

Normal human pancreatic tissue samples were obtained from patients undergoing elective pancreatic surgery for left-sided pancreatic tumours, duodenal tumours, non-obstructive right sided cancer resections in patients with no history of jaundice or chronic pancreatitis as described in (Murphy, Criddle et al. 2008) at the Royal Liverpool University hospital (sampling approved by Liverpool Adult Local Research Ethics Committee [Reference: 03/12/242/A]). On the day before the patient was due to undergo the appropriate pancreatic surgery, written informed consent for sampling of macroscopically normal pancreas from the transection margin during the operative procedure was obtained. During resection a 1-3 cm^3 piece of normal pancreas was taken from the transection margin of the remaining pancreas (figure 2.1) with a new scalpel blade to limit gross macroscopic cell damage. The sample was immediately washed by transfer between two 50 ml tubes of ice-cold extracellular solution to remove debris and blood products such as neutrophils or macrophages which can potentially induce oxidative stress and tissue damage. The sample was added to a third tube containing ~ 50 ml ice-cold extracellular solution plus soya bean trypsin inhibitor, protease inhibitors and sodium pyruvate (Criddle, Murphy et al. 2006) and immediately transported on ice to the laboratory to commence isolation within 10 minutes of sampling. The process of human PAC isolation was similar to murine acinar isolation - however microscopic confirmation of human PACs using transmitted light microscopy was always performed before starting the experiment, as the quality of the cells varied between patients and experiments were conducted on high quality samples only. As described by (Murphy, Criddle et al. 2008) cells

that look damaged morphologically were positive for BZiPAR fluorescence indicating massive premature trypsin activity, Experiments were performed at room temperature and (23-25^{o} C, except when indicated) and cells were used within 4 hours of isolation.

A



B

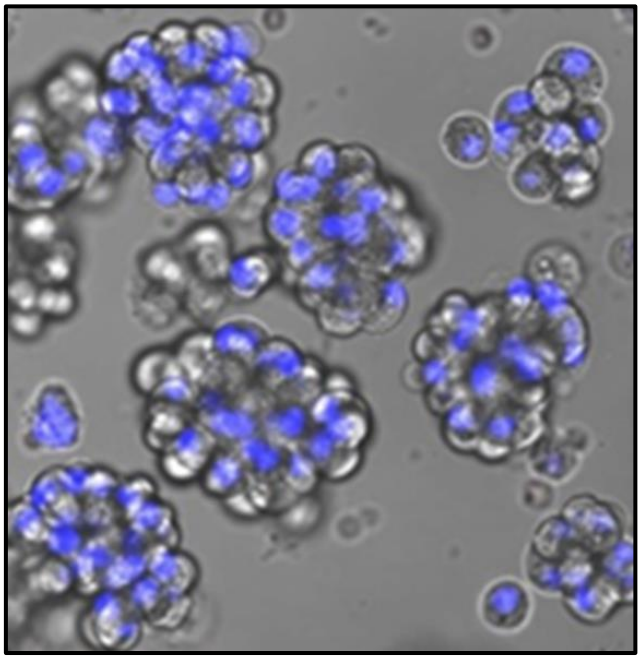


Figure 2.1 - Isolation of human PACs

Magnified image of gross normal human pancreatic tissue sample obtained intra-operatively (A). Representative transmitted light, confocal microscopy image showing clusters of freshly isolated human PACs loaded with fluorescent dye Hoescht used to stain nuclei (B).

2.4 Confocal fluorescence microscopy

2.4.1 Acinar cell intoxication

Isolated PACs were continuously perfused with a supramaximal concentration of 500 µM TLCS (Sigma, Gillingham, UK), 10 nM CCK (Sigma, Gillingham, UK) or 100 µM POAEE (Cambridge Bioscience UK, in 5% ethanol) directly dissolved in Na HEPES for mitochondrial membrane potential ($\Delta\psi_m$), NAD(P)H and cytosolic calcium assays as described in section 2.43 . Cells were pre-treated with MPTP inhibitors for 30 minutes prior to addition of toxins.

For cell death assays, PACs were incubated with 500 µM TLCS dissolved in Na HEPES or 100 µM POAEE (Cambridge Bioscience UK) dissolved in 5% ethanol to induce *in vitro* abnormalities typical of AP *in vivo* (Criddle, Murphy et al. 2006). Cells were co-treated with MPTP inhibitors and pancreatic toxins for 30 minutes prior to imaging.

2.4.2 Cell imaging

Acinar cells were loaded with appropriate dye(s) (table 2) and imaged using state-of-the-art confocal microscopes including Zeiss LSM 510 (for $\Delta\psi_m$, NAD(P)H and cytosolic calcium measurements) and LSM710 (for necrotic cell death assays), equipped with multiple laser lines and an automated stage (710) for multi-field, time-lapse imaging. In all cases cells were visualized with a C-Apochromat 63X water immersion objective. About 150-180 µl of PACs suspended in Na HEPES were placed in a microplate and healthy looking duplets or triplets of acinar cells with good loading of fluorescent dyes were

selected for recording of $\Delta\psi_m$, NAD(P)H and Ca^{2+}. Imaging parameters for cell death assay using confocal microscopy are further described in section 2.44.

2.4.3 Mitochondrial membrane potential, NAD(P)H and cytosolic Ca^{2+} measurements

To assess mitochondrial function live acinar cells were loaded with 50 nM tetramethylrhodamine methyl ester (TMRM; Invitrogen, Paisley, UK; excitation 543 nm, emission >550 nm), a dye used to measure $\Delta\psi_m$ (Voronina, Barrow et al. 2004). Similarly, cells were loaded with Fluo-4 (Invitrogen, Paisley, UK; excitation 488 nm, emission 505 nm) by incubation in solution containing 3 µM Fluo-4AM to measure changes in cytosolic calcium. Simultaneous measurements of NAD(P)H auto-fluorescence (excitation 351 nm, emission 385-470 nm) were done to assess mitochondrial metabolism (figure 2.2). Typically cells were pre-treated for 30 min with test compounds or vehicle prior to the start of the experiment. After assessing baseline fluorescence levels (Fo), cells were stimulated with 500 µM TLCS, 10 nM CCK or 100 µM POAEE, for 10 minutes and then treated with 10 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP; Sigma, Gillingham, UK), as a positive control. Cells were imaged every 5-10 seconds under confocal microscopy for measurement of $\Delta\psi_m$, NAD(P)H and calcium in the absence or presence of MPTP inhibitors. TMRM, NAD(P)H and Fluo-4 fluorescent signals were normalized versus baseline fluorescent level (F/Fo).

Table 2 - Fluorescent indicator loading protocols

Flouresect indicator	Loading conc.	Loading time (min)	Excitation (nm)	Emission (nm)	Target organelle / species
TMRM	50 nM	25-30	543	580-680	Mitochondria $\Delta\psi_m$
Fluo-4 AM	3µM	25-30	488	500-550	Cytosolic calcium
Propidium iodide	1 µM	25-30	488	630-693	Nucleus (cell impermeant)
Hoechst	1 µM	25-30	361	486	Nucleus (cell permeant)

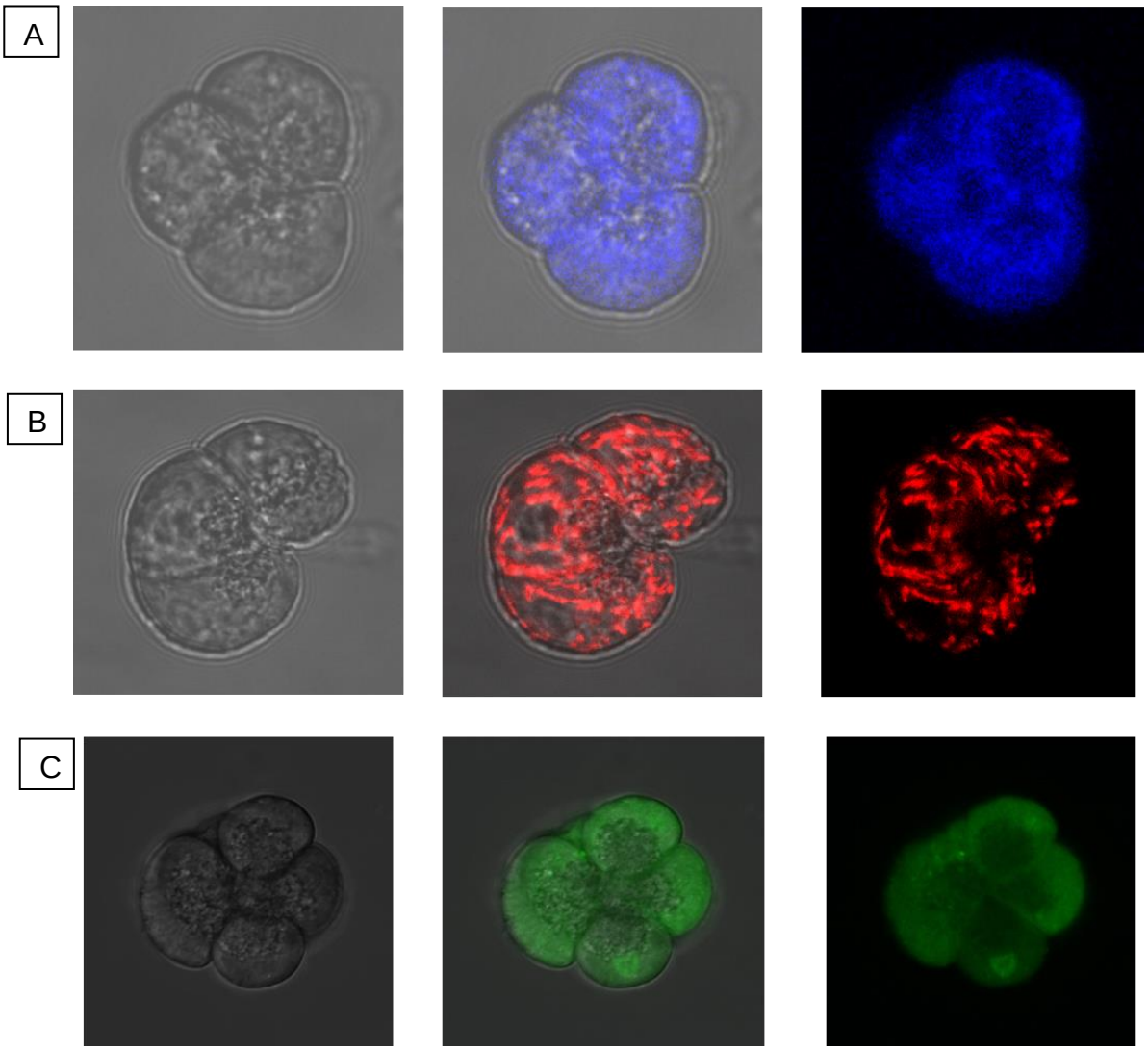


Figure 2.2 - Confocal fluorescence microscopy of isolated PACs

(A) Transmitted light and confocal images of triplet of mouse PACs showing NAD(P)H autoflourescence (blue). (B) Transmitted light and confocal images of PAC doublet, loaded with TMRM (red) demonstrating mitochondrial distribution within PAC. (C) Transmitted light and confocal images of pancreatic acini, loaded with Fluo-4AM (green) showing calcium distribution within PAC.

Note: All images acquired under physiological conditions

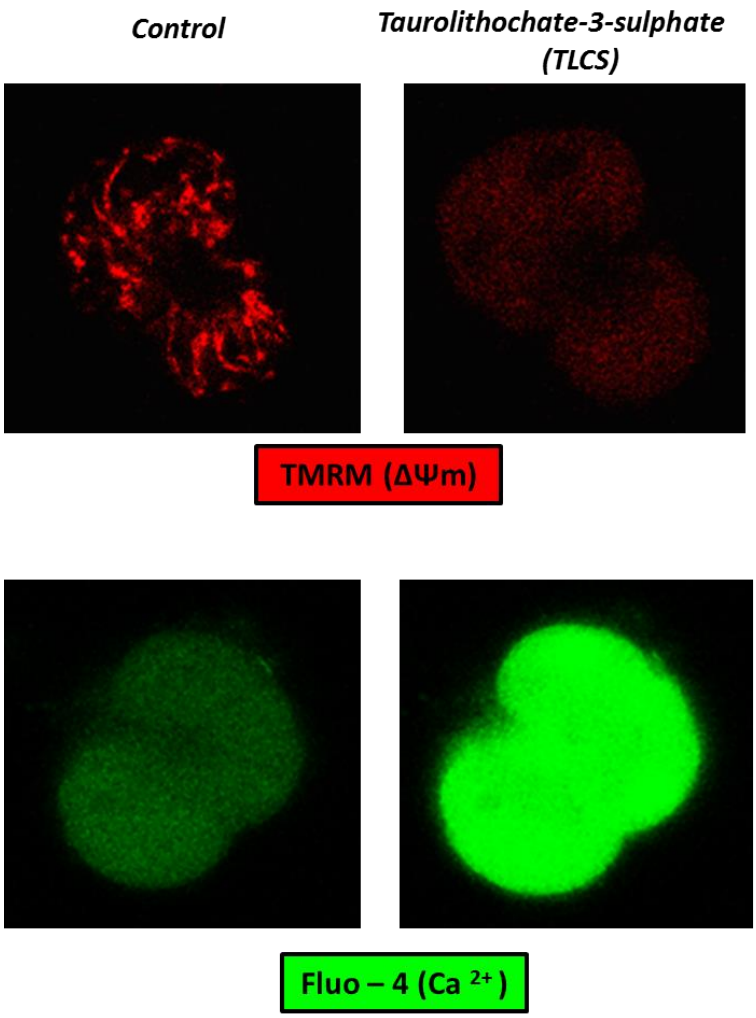


Figure 2.3 - Effects of TLCS stimulation on $\Delta\psi_m$ and calcium

Confocal images of doublet of murine PACs loaded with TMRM (red) and Fluo-4 (green) showing changes to $\Delta\psi_m$ and Ca^{2+} in response to TLCS stimulation respectively. Exposure to TLCS leads to loss of $\Delta\psi_m$ indicated by loss of TMRM fluorescence signal (A) and a corresponding increase in Fluo-4 fluorescence signal indicating cytosolic calcium overload (B).

2.4.4 Necrotic cell death assay

In order to assess activation of necrotic cell death pathway, 1 μM propidium iodide (PI; excitation 488 nm, emission 630-693 nm) was used to evaluate plasma membrane rupture (Criddle, 2006). Isolated PACs from each animal were divided into the following groups:

(i) control – PAC only; acinar cells incubated with Na HEPES for 30 minutes and imaged,

(ii) PAC + 500 μM TLCS; acinar cells incubated with 500 μM TLCS for 30 minutes and imaged,

(iii) PAC + 500 μM TLCS + MPTP inhibitors simultaneously and,

(iv) in case of assessment of novel CypD inhibitors; PAC + small molecular CypD inhibitor (for evaluation of toxicity of test compound).

Incubation time was 30 minutes for each group and all experiments were conducted at room temperature. Similarly acinar cells were also incubated with 100μM POAEE in the presence or absence of test compound and imaged. Sixteen randomly selected fields of view were taken of each mouse isolate (figure 2.4) and the total number of cells displaying PI uptake were counted per field to give a percentage ratio for each field, averaged across fields, and converted to a mean +/- SEM for a minimum of three mice and two or three humans samples per experimental group. The experiments were performed in a blinded fashion, such that the observer choosing the fields and the observer undertaking image analysis was blinded with respect to the treatment groups.

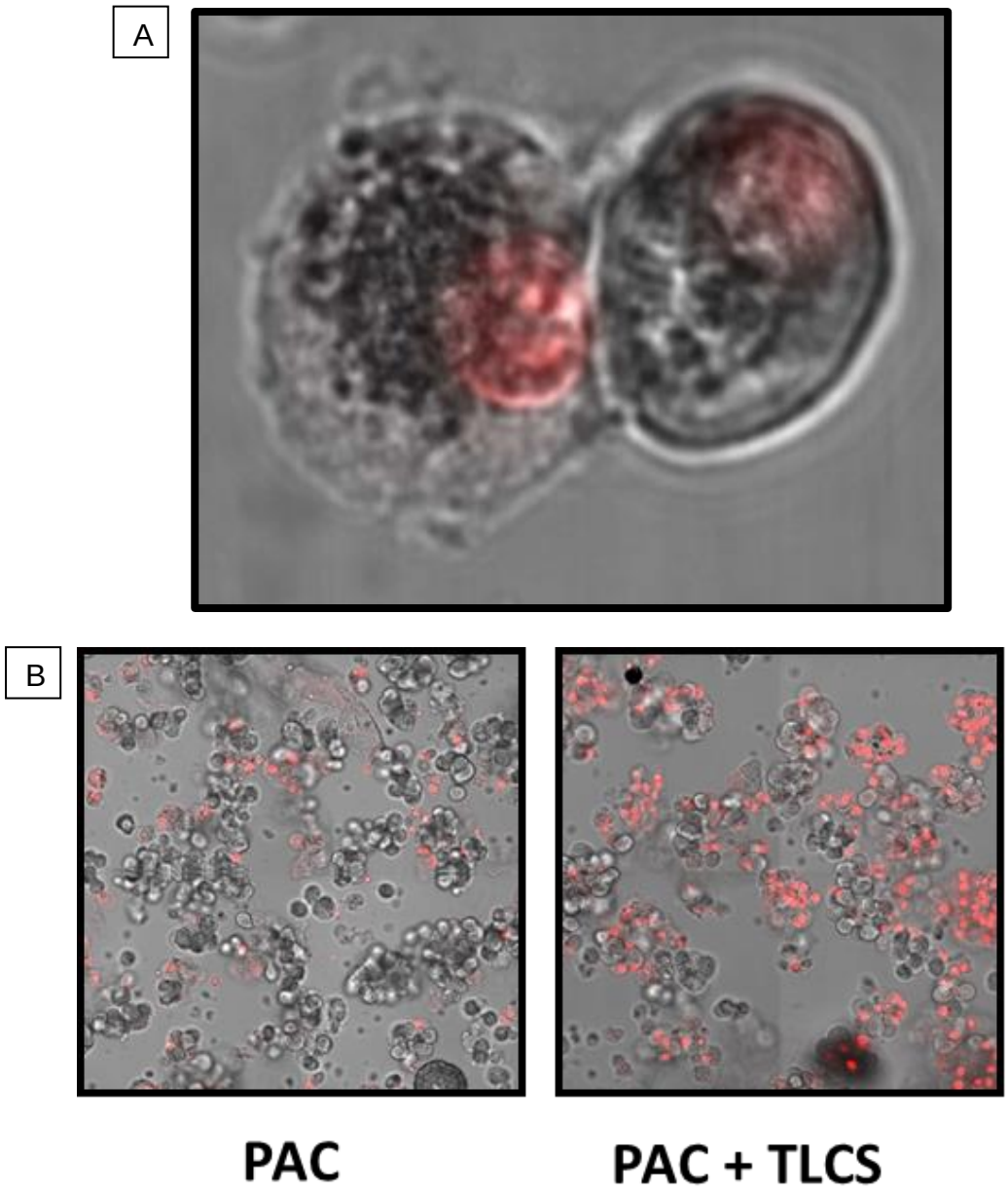


Figure 2.4 - Necrotic cell death assay

(A). Representative image of murine PAC doublet showing uptake of propidium iodide (PI), a membrane impermeable dye (shown in red) indicating necrotic cell death pathway activation. (B). Incubation of PAC with TLCS (500 μM) at room temperature for 30 minutes causing plasma membrane rupture and increase in PI uptake, compared to control.

2.5 Genotyping for Ppif null mice

A standard Polymerase Chain Reaction (PCR) assay for genotyping the Ppif animals was used. After the tail prep, the genomic DNA was suspended in 75 µl of TE (Tris and EDTA) buffer and hydrated. The sample was diluted in a ratio of 1:20 and 1 ml of this dilution was used for the PCR (50 ml reaction volume). The three primers used in a single reaction were:

Exon3-F: CTC TTC TGG GCA AGA ATT GC

Neo-F: GGC TGC TAA AGC GCA TGC TCC

Exon4-R: ATT GTG GTT GGT GAA GTC GCC

Reaction conditions: 95°C for 3 min

95°C for 0.5 min 35 cycles

56°C for 0.5 min

72°C for 1 min

72°C for 10 min

2.6 Synthesis of recombinant cyclophilin D

Human CypD clone (LIFESEQ7721956) was bought from Thermo Scientific Open Biosystem. By using the forward primer 5'-CCGTGGATCCATGGGGAACCCGCTCGTGTAC-3' and the reverse primer 5'-CCGT CTCGAGTTAGCTCAACTGGCCACAGTC-3', the PCR product was sub cloned into the vector pET28b (+) (NOVAGEN) between the BamH1 and Xho1 sites to obtain the expression of pET28b-CypD. Sequencing was carried out to confirm the insertion. In order to increase the solubility of the protein for

crystallographic studies, a mutation of Lys133 into Ile was introduced on the CypD gene.

Expression was conducted in E. coli BL21 cells. Four 2 litre flasks each with 500ml culture media were inoculated with an overnight culture of freshly pET28b-CypD transformed cells supplemented with 30µg/ml kanamycin. The bacteria at 37 °C were grown until the OD600 reached 0.6, and isopropylthio-β-D-galactoside (IPTG) was added to final concentration of 1mM to induce HisTag-CypD expression at 30 °C overnight. Bacteria were harvested and re-suspended in 25mM Tris-HCl pH 7.8, 500mM NaCl and 20mM imidazole buffer and were lysed with a French press at 60 MPa. The lysate was centrifuged and supernatant was collected. HisTag-CypD protein was purified by using Ni Sepharose HisTrap FF column (GE Healthcare Life Sciences).

2.7 Surface Plasmon Resonance assay for screening of small molecules

The Surface Plasmon Resonance (SPR) technology-based Biacore X100 (GE Healthcare) was used for screening of small molecules based on their interaction with CypD. All experiments were carried out using buffer containing Tris-HCl pH 7.6, NaCl 150mM, 0.05% surfactant p20 and 5% of dimethyl sulfoxide (DMSO) as a running buffer at a constant flow rate of 30 µl/min at room temperature. The protein, CypD, was immobilized directly and covalently on hydrophilic carboxymethylated dextran matrix of the CM5 chip sensor chip (BIAcore) by using the standard primary amine coupling. The protein (25 µg/ml) was immobilized on CM5 sensor ship to achieve ~2500 response units. All data

analyses were carried out using BIA evaluation software and the sensorgrams were processed using the automatic correction method for nonspecific bulk refractive index effects. The kinetic analyses of the ligand-binding to the protein were performed based on the 1:1 Langmuir binding fit model according to the procedure described in the software.

2.8 Induction of experimental acute pancreatitis

2.8.1 Caerulein induced experimental acute pancreatitis

The first model tested was hyperstimulation with caerulein, a cholecystokinin analogue, which is the most widely used model of experimental acute pancreatitis (CER-AP), the severity of which can be graded depending on the extent of hyperstimulation (Pandol, Saluja et al. 2007). The clinical parallel of this model is hyperstimulation-induced AP by scorpion bites (notably *Tityus species*), frequent in the West Indies and South America, or by organophosphate insecticides. Compounds in scorpion venom and organophosphates irreversibly inhibit cholinesterase and provoke excessive cholinergic stimulation. A standard model of seven, hourly intraperitoneal injections of 50 µg/kg caerulein (dissolved in saline solution) was used, with controls receiving saline alone; sacrifice was made 12 hours after the first injection. Three doses (1mg/kg, 3mg/kg or 10 mg/kg) of liposomal preparation of TRO40303 were administered after the third caerulein injection. The most effective dose was tested in subsequent models.

2.8.2 Bile acid induced acute pancreatitis

The next model was the bile acid-induced murine acute pancreatitis (TLCS-AP), similar to gallstone-induced clinical AP, which results from retrograde passage of bile into the pancreatic duct when gallstones get impacted at the sphincter of Oddi. A retrograde injection of 3 mM TLCS was administered into the pancreatic duct, perfused for 10 minutes and 3mg/kg of TRO40303 was administered intraperitoneally one hour after the TLCS injection. In order to

ensure appropriate technique and adequate pancreatic duct infusion in each case, characteristic ballooning and blue stippling of the pancreas was observed for each mouse. Controls had the same procedure without ductal infusion; sacrifice was made 24 hours later. The previously described technique was modified (Laukkarinen, Van Acker et al. 2007) and intraperitoneal anaesthesia was substituted with gaseous anaesthesia using a combination of O_2, N_2O and isoflurane to facilitate a more controlled induction and recovery process. Analgesia was administered prior to induction; mice were given 1mg/kg bodyweight of buprenorphine subcutaneously. Following laparotomy, the common duct was cannulated via trans-duodenal puncture using 30 guage blunt needle on the anti-mesenteric border of the duodenum. The needle was secured in position with a 10/0 prolene stitch and micro vessel clip applied at the liver hilum to ensure preferential perfusion of the pancreas rather than biliary tree. The solution (dissolved in Na HEPES solution with methylene blue ~ 300 µM) was perfused at a rate of 5 µl/min for 10 minutes using infusion pump (Harvard apparatus) and blue discolouration of the pancreas was monitored to ensure consistent pancreatic infusion and delivery of TLCS. The needle was removed at the end of infusion period followed by closure of the duodenal defect, abdomen and mouse recovered.

2.8.3 Alcoholic acute pancreatitis

The third model of experimental murine AP with a clinical parallel was tested by intraperitoneal injection of ethanol and palmitoleic acid (FAEE-AP), a fatty acid that combines with ethanol in the pancreas to form fatty acid ethyl esters, the principal agents of PAC injury from ethanol excess (Huang, Booth et al. 2014).

Mice received two intraperitoneal injections of 1.32 g/kg ethanol and 1.5 mg/kg palmitoleic acid (dissolved in peanut oil), each one hour apart; to induce AP. Controls received intraperitoneal saline injections and in treatment group, 3mg/kg of TRO40303 was administered intraperitoneally one hour after the last injection. Humane killing was performed 24 hours after the first injection and analgesia administered (1mg/kg bodyweight of buprenorphine subcutaneously) prior to induction of AP and 12 hours after the first injection.

All experiments complied with a Project Licence, under the terms of the Animals (Scientific Procedures) Act 1986, authorising the proposed experiments, granted by the Home Secretary UK. TRO40303 stock solution in liposomes (20 mg/ml) was diluted into saline to prepare 2.5 fold concentrated working solutions (0.4, 1.2 and 4 mg/ml, for the three doses 1, 3 and 10 mg/kg, respectively), which were injected intraperitoneally using a constant volume of 2.5 ml/kg.

Standard times for assessment after the start of the induction of EAP were 12 hours after the first injection of CER-AP and 24 hours after the first injection of TLCS-AP and FAEE-AP. At these time points the local and systemic disease is well established, and various parameters most severe, representing the time point when a difference between control and treatment groups is most likely to be found. Before sacrifice (at 12 hours after the first injection of CER-AP or 24 hours after the first injection of TLCS-AP and FAEE-AP), blood samples were harvested on Lithium Heparin tubes (VenoJect®, TERUMO®) and centrifuged immediately for 15 minutes at 2.500 rpm at 4°C. Pancreata were then immediately isolated for extract preparation and histology

slides. Lungs were also isolated for extract preparation. Each group of mice at each time point undergoing each protocol were constituted of six animals.

2.9 Biochemical parameters of severity of experimental acute pancreatitis

2.9.1 Serum amylase, IL-6 and pancreatic trypsin activity determination

Amylase levels were tested using a kinetic method by Roche automated clinical chemistry analysers. IL-6 was measured using enzyme-linked immunosorbent assay (ELISA) - Quantikine from R&D systems. Trypsin activity was measured with a fluorogenic assay, using Boc-Gln-Ala-Arg-AMC substrate converted by trypsin to a fluorescent product (excitation 380 nm, emission 440 nm).

2.9.2 Myeloperoxidase assay

Myeloperoxidase (MPO) activity, indicative of neutrophil infiltration, was measured in pancreas and lungs (Dawra, Ku et al. 2008). For measuring MPO activity, 20 µl of extract was added into the assay mix consisting of 200 µl phosphate buffer (100 mM, pH 5.4 with 0.5% HETAB), 20 µl 3,3′,5,5′-tetramethylbenzidine (TMB) 20 mM in DMSO. This mixture was incubated at 37^{o}C for 3 minutes, followed by addition of 50 µl of H_2O_2 (0.01%) which was further incubated for 3 minutes. The difference of absorbance between 0 minutes and 3 minutes at 650 nm was calculated by a standard curve triggered by human MPO.

2.10 Histopathological parameters of severity of experimental acute pancreatitis

Pancreatic tissue was fixed in formaldehyde and standard haemotoxylin and eosin sections were prepared. Scoring of oedema, leucocyte infiltration and necrosis (0-3) was undertaken by two independent, blinded investigators (free marginal $\kappa = 0.75$) in X 10 high power fields/slide/mouse (Wildi, Kleeff et al. 2007) [Table 3]. X 200 magnification was used throughout. Scores were summated then means +/- SEM calculated for mice in each experimental group.

Table 3 - Criteria used for histological analysis of severity of EAP

Condition	Score	Indication
Oedema	0	Absent
	1	Focally increased between lobules
	2	Diffusely increased
	3	Acini disrupted and separated
Inflammatory cell infiltrate	0	Absent
	1	In ducts (around ductal margins)
	2	In the parenchyma (<50% of the lobules)
	3	In the parenchyma (>50% of the lobules)
Acinar necrosis	0	Absent
	1	Periductal necrosis (<5%)*
	2	Focal necrosis (5–20%)
	3	Diffuse parenchymal necrosis (20–50%)

- Criteria described by Van Laetham and colleagues

*Approximate percentage of cells involved per field examined.

2.11 TRO40303 plasma level measurements

Preliminary pharmacokinetic (PK) analysis was performed at Trophos with the liposomal intraperitoneal administration in C56B7 naive mice at 3 and 10 mg/kg. A maximum of 0.25 mL of blood was collected into lithium heparin tubes at 15 and 30 minutes, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr after drug administration (3 mice per time point). The blood samples were cooled on ice and plasma samples were prepared within 60 minutes of sampling by centrifugation at 1500 g at 4˚C for 10 minutes and stored at -20°C until analysis.

For pancreata level measurements, TRO40303 was administrated intraperitoneally to mice at the dose of 3 mg/kg in liposomes, pancreata were isolated at 15 min, 2hr and 24hr (3 mice per time point) and washed with saline. Samples were frozen and stored at -20°C until analysis.

TRO40303 level was also assessed both on the plasma and pancreata in the cearulin model at the doses of 1, 3 and 10 mg/kg of TRO40303 15 minutes after TRO40303 administration. Plasma was harvested and stored at -20°C in eppendorf for shipment to Trophos for bio-analysis.

Thawed samples were extracted with acetonitrile, centrifuged, purified on SPEC C2 cartridge (Varian) and analysed by HPLC-MS/MS along with calibration standards. Analysis was carried out using the Alliance 2695 (Waters) system interfaced to an API Quattro Micro (Waters) MS detector. Waters Quan Lynx software was used for calculations.

2.12 Statistical analyses

Biological data were distributed normally and therefore presented as mean +/- SEM. Non-normally distributed variables (section 3.3) are presented as median and interquartile range (IQR). Statistical evaluation was performed using OriginPro 9 (Origin Lab corporation, USA). Two-tailed student's t-test (two groups) and ANOVA (more than two groups) were performed for parametric data. Unpaired t-test was used to calculate the difference in means of independent samples. Null hypotheses were rejected at the 5% significance level and P values <0.05 were considered significant. Although no formal calculation of sample size was undertaken for experiments involving experimental acute pancreatitis, at least six animals were included in each group, which is considered an adequate sample size by many researchers (Charan and Kantharia 2013) and has been the standard sample size in peer reviewed publications from our group (Huang, Booth et al. 2014, Mukherjee, Mareninova et al. 2015, Wen, Voronina et al. 2015, Javed, Wen et al. 2018). The kappa statistic was calculated to test interrater reliability of the two independent reviewers undertaking blinded assessment of histological markers of severity in pre-clinical models of experimental acute pancreatitis .

3. Chapter 3 – Systematic review and qualitative assessment of studies evaluating agents tested for treatment of experimental acute pancreatitis

3.1 Introduction

Experimental murine models of AP have been used to investigate the molecular mechanisms underlying the disease process and screen potential therapies for testing in humans (Gorelick and Lerch 2017). Rodents have several advantages over other model organisms; the mouse genome shares similarity with the human genome (99%), provides an appropriate genetic and molecular toolbox to produce relevant knockout species and the animal's small size facilitates high throughput studies making it a cost-efficient model. Although a number of agents have shown efficacy in pre-clinical studies, it has not been possible to translate the experimental therapeutic results into clinical practice in human AP. This may be due to the difficulty in designing clinical studies capable of giving reliable, statistically significant answers regarding the benefits of the various proposed therapeutic agents previously tested in experimental settings. It is however, important to try and understand other factors that may contribute towards the lack of translation of potential therapeutics from bench to bedside. Drug development for stroke is a relevant example where the efficacy of drugs in pre-clinical models has failed to translate into clinical trials; a reported attrition rate of 99% at the stage of clinical trial alone, since the animal experiments did not model human disease with sufficient fidelity to provide a useful guide for translation (Sena, van der Worp et al. 2007). These pitfalls have been addressed by a better understanding of pathophysiology, recognition for the need of early intervention, establishment of stroke units and a multidisciplinary approach in the form of recommendations from the Stroke Therapy Academic Industry Round Table to suggest guidelines for the preclinical evaluation of

neuroprotective drugs (Stroke Therapy Academic Industry 1999). A similar approach may need to be adopted by the pancreatic community for developing a treatment of AP.

The following systematic review and qualitative assessment of all studies evaluating agents used for the treatment of AP in murine models of experimental pancreatitis provides an up to date analysis of all agents that have been tested pre-clinically and aims to identify any factors that may contribute towards this significant translational gap and for the clinical treatment of AP.

3.2 Methods

3.2.1 Study Identification

Multiple electronic databases including Pubmed, Medline, Embase and Cochrane library were searched from the time of inception to December 2015 by two independent reviewers using Medical Subject Headings (MeSH) terms ‘acute pancreatitis’, ‘treatment’ and ‘therapy’ to identify the studies (figure 3.1). The search process was augmented by manual review of the lists of references cited in identified studies and review articles, by scanning abstracts from conference proceedings and contacting investigators if required.

3.2.2 Inclusion Criteria

- Studies published in the English language

- Studies published as full reports
- Pre-clinical studies evaluating efficacy of an agent in murine models of EAP only
- Studies aimed at evaluating therapeutic potential of an agent rather than patho / physiology of AP

Studies evaluating combination treatments or agents used for treatment of complications were excluded. Agents were classified according to their putative mechanism of action into the following categories:

- Anti-inflammatory agents
- Enzyme inhibitors
- Anti-oxidants
- Secretion inhibitors
- Agents affecting blood flow and microcirculation
- Eicosanoids
- Others (miscellaneous and multiple actions)

3.2.3 Quality assessment

A newly devised scoring system was formulated in consultation with two internationally renowned pancreatologists (Professor Robert Sutton, UK and Professor Markus Lerch, Germany) and used for qualitative assessment of each publication. The scoring system comprised a checklist of 10 points that included; adequate number of animals per group, reference to posology, clinical relevance and number of models of AP, prophylactic vs therapeutic administration of treatment, adequate description of husbandry, biochemical

and histological markers of severity and evaluation of other organs. Each study was scored by two independent reviewers. In case of a lack of consensus, a third reviewer was invited to score the study and to adjudicate. Studies fulfilling the following criteria were analysed in further detail including a semi-quantitative assessment of whether an agent reduced the biochemical or histological markers of severity by more than 50% or not, as these were considered to be the most clinically relevant and objective parameters of assessment.

(i) Adequate number of animals ($n \geq 6$),

(ii) clinically representative model (caerulein, bile acid, ethanol induced or pancreatic duct ligation),

(iii) treatment administered therapeutically i.e after induction of pancreatitis. In caerulein model treatment was considered therapeutic after the 3rd caerulein injection in mice or 4 hours of caerulein infusion in rats. In all other models therapeutic administration defined as commencement of treatment after completion of induction of EAP.

(iv) Blinded assessment of pancreatic histology.

3.3 Results

3.3.1 Literature review process

The flow diagram depicting the literature search, subsequent study identification and inclusion process is shown as figure 3.1. Essentially, 771 studies were identified using appropriate and relevant MeSH terms. Six hundred and sixty were found to be eligible. Out of these, 230 were excluded as they were testing a combination of treatments, primarily aimed at treating complications of AP rather than pancreas or were mechanistic and therefore not testing efficacy of a therapeutic agent. Finally, 430 studies were included in the qualitative synthesis and categorised according to the agent's putative mode of action.

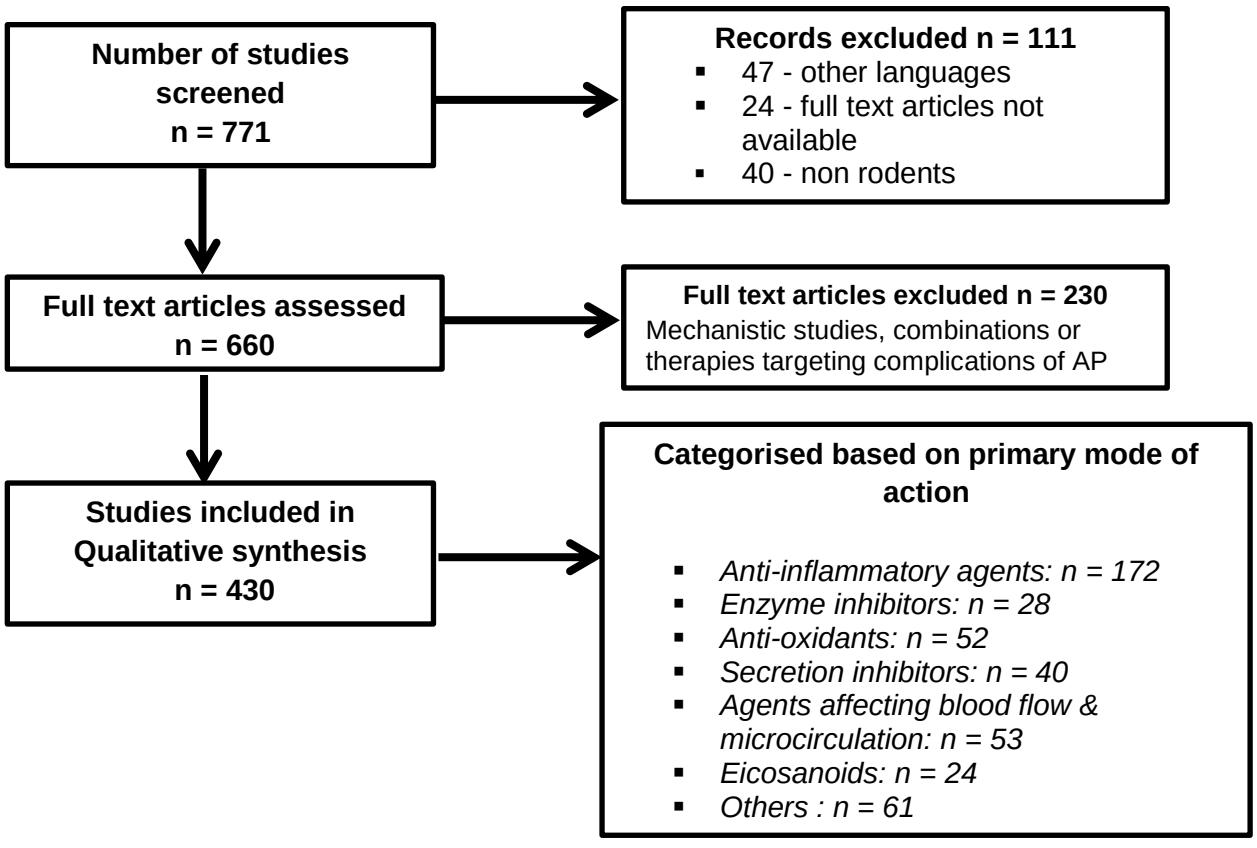


Figure 3.1 - Flow diagram describing literature review process

3.3.2 Experimental models of acute pancreatitis and variation in pre-clinical experimental design

A wide range of murine experimental models of AP have been used to test the therapeutic efficacy of potential AP treatments which have been summarised in table 4. Caerulein and bile acid induced AP were by far the two most common models tested in experimental studies. A majority of experimental studies have been conducted on rats (74.2%) and efficacy of agents have been tested prophylactically (58.8%) – figure 3.2 A,B. In 28.8% of publications, treatment was administered therapeutically and in 53 studies, both prophylactic as well as therapeutic effects of an agent were evaluated (figure 3.2B). There was significant variation in the timing of therapeutic administration of treatment (figure 3.3A) as well as the severity of experimental pancreatitis in CER-AP and TLCS-AP (figure 3.3 B-D). The vast majority of studies were undertaken using a single model of EAP, less than 10% evaluated the therapeutic potential of a test compound in two models and only ten publications used more than two models of EAP (figure 3.2C).

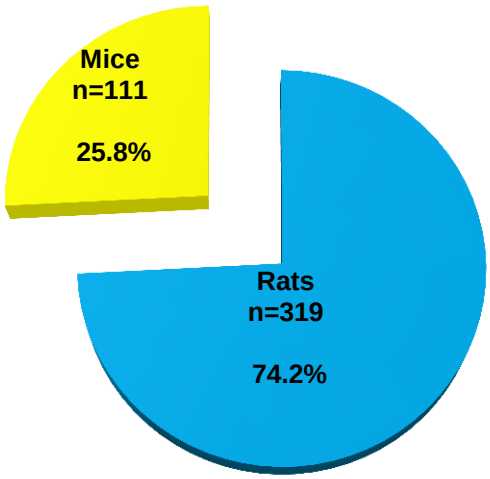
Table 4: Experimental models of acute pancreatitis

1. Caerulein induced pancreatitis (Niederau, Ferrell et al. 1985)*	***2. Bile acid infusion model of pancreatitis*(Wittel, Wiech et al. 2008)***
3. Pancreatic duct (PD) ligation*(Walker, Winterford et al. 1992)	***4. Bile acid infusion combined with PD ligation*(Osman, Lausten et al. 1999)***
5. Intraductal contrast infusion (Folch-Puy, Granell et al. 2006)	6. Ethanol induced (Hirano 1994)
7. Closed duodenal loop (Nevalainen and Seppa 1975)	8. L- Arginine induced (Mizunuma, Kawamura et al. 1984)
9. Caerulein combined with bile acid (Schmidt, Rattner et al. 1992)	10. Choline deficient ethionine (CDE) – diet induced (Lombardi, Estes et al. 1975)
11. Bile acid + trypsin infusion (Grewal, Kotb et al. 1994)	12. Caerulein combined with lipo-polysaccharide (LPS) administration (Ding, Li et al. 2003)
13. Intraductal infusion of TLCS and phospholipase A2 (Hatao 1969)	14. Ischaemia induced (Dembinski, Warzecha et al. 2001) (Ceranowicz, Dembinski et al. 2008)
15. Intraductal trypsin infusion (Lankisch, Winckler et al. 1974)	16. Ethanol induced combined with caerulein (Yuasa, Irimura et al. 1998)
17. Traumatic pancreatitis (Ren, Luo et al. 2012)	***18. Alcoholic pancreatitis (Huang, Booth et al. 2014)***

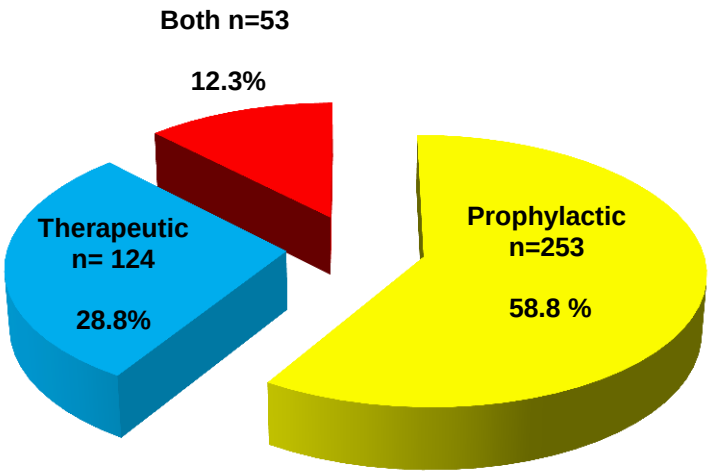
* ***Highlighted models were considered clinically relevant***

Figure 3.2: Variation in experimental models of acute pancreatitis

A: Species



B: Administration of treatment



In caerulein model therapeutic administration of treatment defined as:

- *After the 3rd caerulein injection in mice*
- *After 4 hours of intravenous infusion in rats*

In all other models therapeutic administration defined as commencement of treatment after completion of induction of experimental acute pancreatitis

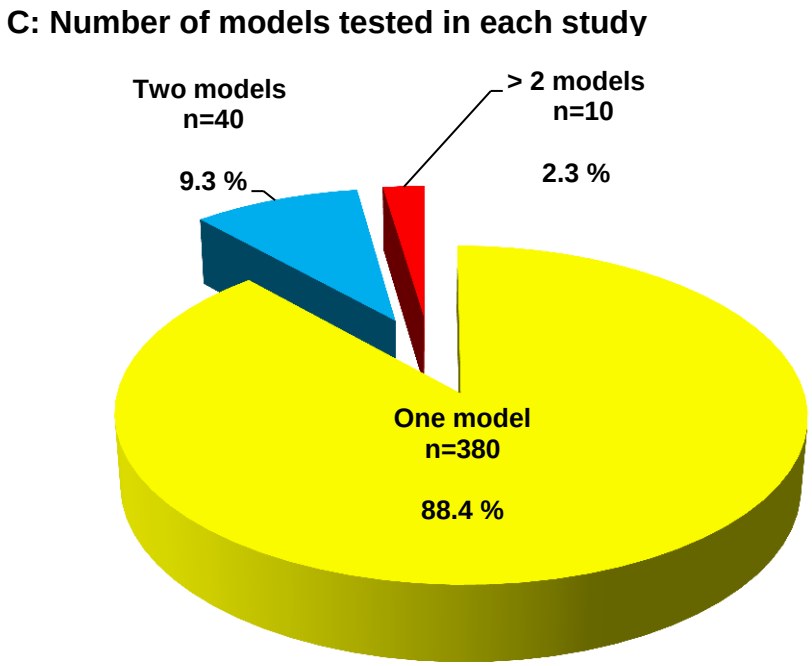
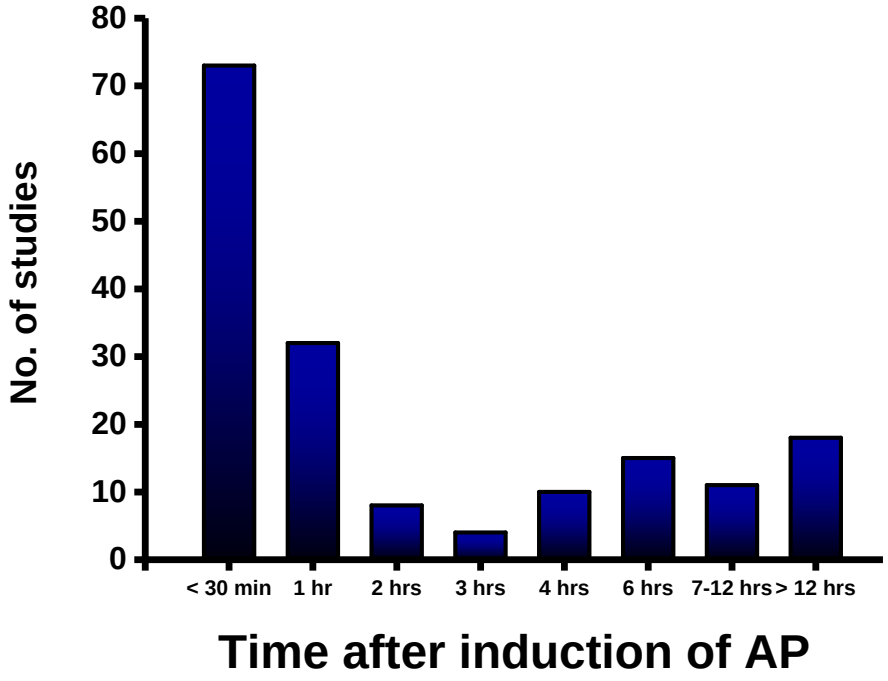


Figure 3.2 - Variation in protocols of EAP models

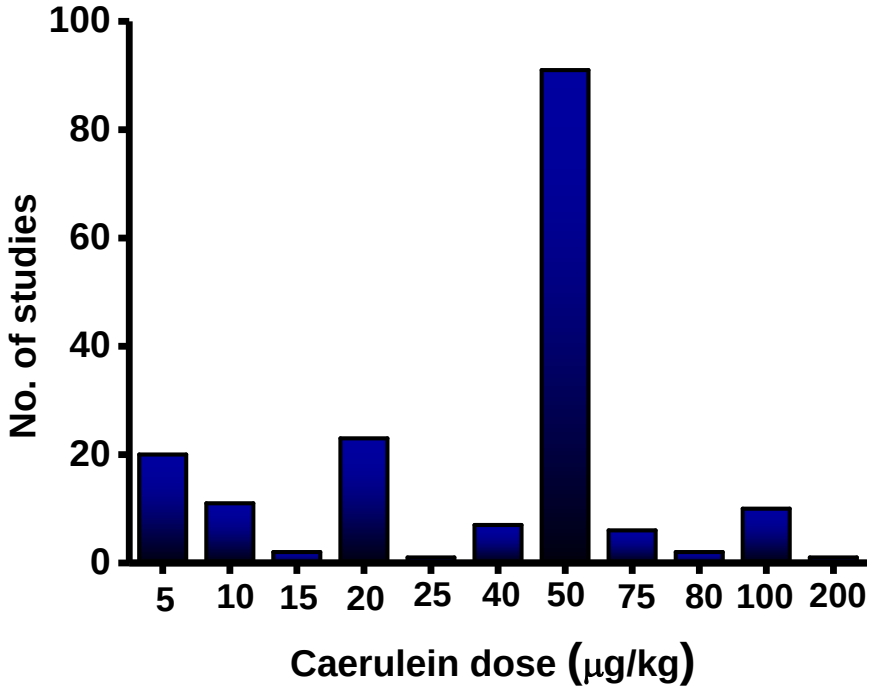
Pie chart showing the different species used in EAP (A), timing of treatment in pre-clinical studies (B) and number of models tested in individual studies (C).

Figure 3.3: Variation in toxicity of experimental models of acute pancreatitis

A: Variation in starting of therapeutic treatment



B: Variation in dose of caerulein



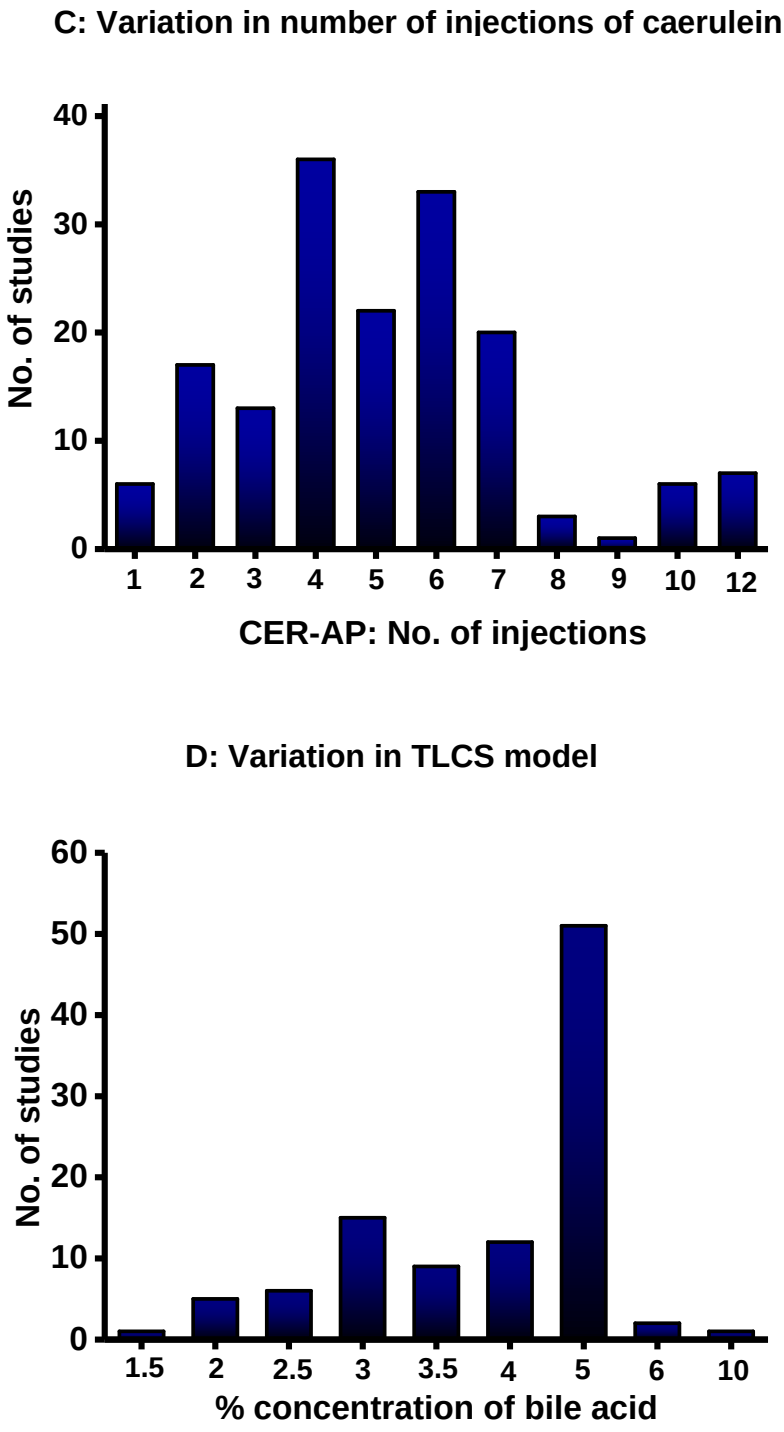


Figure 3.3 - Variation in toxicity of EAP models

Therapeutic administration of testing agent can vary from just after AP induction up to 12 hours post induction (A). Significant variation in the dose (B) and number of injections (C) in CER-AP is also evident. Concentration of bile acid can vary from 1.5%-10% in bile acid induced AP (D).

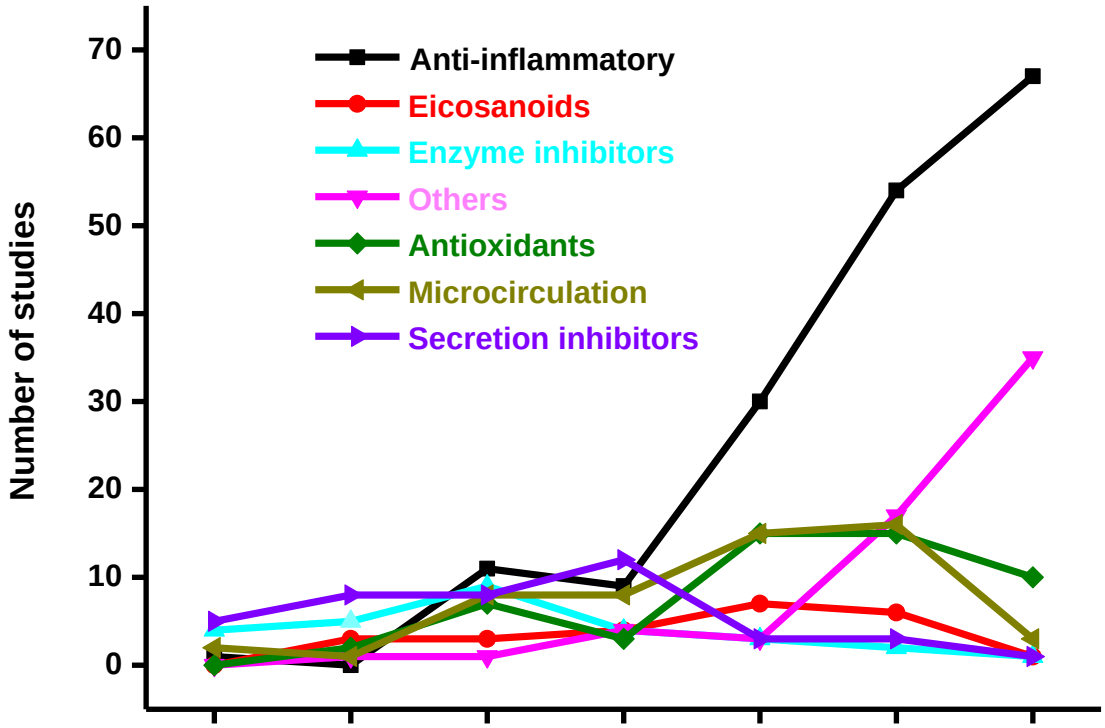
3.3.3 Targets and trends in publications over time

Agents were categorised according to their putative mechanism of action for the purposes of classification. Table 5 shows the number of agents tested within each category in preclinical studies and details of all the compounds that have been tested in murine models of AP are summarised in tables 8-14. Premature digestive enzyme activation is considered to be a cardinal feature of pancreatic injury in pancreatitis, with activated zymogens contributing to intra- and extra-cellular damage. Hence considerable attention has been focused on pharmacological agents that inhibit activated proteases (n=28). It is generally believed that the severity of pancreatitis is determined by events that occur after acinar cell injury. Pancreatic acinar cells synthesize and release cytokines and chemokines, resulting in the recruitment of inflammatory cells such as neutrophils and macrophages. Recruitment and activation of various inflammatory cells leads to further acinar cell injury and causes an elevation of various pro-inflammatory mediators such as TNF α, IL-1, IL-2, IL-6, and other chemokines and anti-inflammatory factors such as IL-10. These inflammatory cells and mediators play a role in the systemic manifestations besides modulating PAC injury. Systemic inflammatory response syndrome (SIRS) is a result of uncontrolled local inflammation and predisposes to multiple organ failure. The suppression of the inflammatory cytokines secretion may reduce pancreatitis intensity and mortality (Bhatia, Brady et al. 2000). Anti-inflammatory agents (n=172) and eicosanoids (n=24) have by far been the most commonly investigated treatment modalities in experimental studies of AP. Reactive oxygen species (ROS) play an important mediator function in the early and late course of AP and have a direct influence on lipids and proteins in

the cell membrane and disrupt their functions. Although the role of ROS is pathogenesis of AP is complex (Booth, Murphy et al. 2011), inhibition of ROS has been tested as a treatment strategy for AP (n=52). Cholecystokinin (CCK) is a gastrointestinal hormone and neurotransmitter located in the digestive tract. Since it stimulates secretion of the pancreatic digestive enzymes via CCK receptors, it is considered to play a key role in the progression and aggravation of AP - it has been targeted as a treatment strategy along with other inhibitors of gastrointestinal secretions (n=40). The release of activated enzymes in pancreatitis results in the activation of plasma proteases of the kallikrein-kinin, complement, coagulation, and fibrinolytic systems and consumption of protease inhibitors. Disturbances within the cascade systems play a crucial role in the clinical outcome and therefore inhibitors of coagulation have been used as a potential treatment modality for pancreatitis (n=53). The trends in published literature regarding treatment strategies tested for the treatment of AP over time are shown in figure 3.4, demonstrating a shift towards targeting of the inflammatory pathway in the last two decades.

Table 5 - Pre-clinical EAP treatment; qualitative assessment

Treatment	Number of pre-studies	Median quality score (IQR)	# of studies fulfilling modified score	# of agents showing > 50 % improvement in biochemical and histological parameters
Anti-inflammatory	172	5.5 (5-6)	25	Benzamide (Yasar, Uysal et al. 2010), IL 10 (Rongione, Kusske et al. 1997) (Chen, Tang et al. 2004), Duchengqi Decoction (Wang, Chen et al. 2012) and Thymosin alpha 1(Wang, Zeng et al. 2015)
Enzyme inhibitors	28	4 (3-5)	2	Ulinastatin (Maciejewski, Burdan et al. 2005)
Anti-oxidants	52	4 (4-6)	9	Caffeic acid phenethyl ester (Buyukberber, Savas et al. 2009) and ozone (Uysal, Yasar et al. 2010)
Secretion inhibitors	40	5 (3-5)	5	None
Microcirculation and blood flow	53	5 (4-6)	6	Activated protein C (Babu, Genovese et al. 2012)
Eicosanoids	24	5 (5-6)	4	None
Others	61	6 (5-7)	15	Pentoxifylline (Matheus, Coelho et al. 2009), GSK7975A (Wen, Voronina et al. 2015), DEB025 and TRO40303 (Mukherjee, Mareninova et al. 2015), Obestatin (Bukowczan, Cieszkowski et al. 2016) and polyenoylphosphatidylcholine (Li, Wu et al. 2015)



	1981-85	1986-90	1991-95	1996-2000	2001-05	2006-10	2011-15
Number of studies	12	20	47	44	76	113	118
Median score (IQR)	3(2.5-3)	4 (2.5-5)	4 (3-5)	5 (4-6)	4 (4-6)	5(5-6)	6 (5-7)

Figure 3.4 - Trends in publications over time

After an initial surge of research involving anti-secretory agents, enzyme inhibitors, compounds affecting blood flow and anti-oxidants; researchers seem to have focused on evaluating anti-inflammatory agents and compounds with multiple actions during the last 15 years.

3.3.4 Quality assessment

Median quality score for all the studies included in the analysis was 5 (IQR: 4-6). The percentage of each reported parameter of the modified scoring system is shown in table 6. Most studies were undertaken in an adequate number of animals, on a clinically relevant model and provided adequate description of approved animal husbandry including board / home office or other standards. Posology was adequately addressed in 45% of the studies, 52% of the studies had evaluated two or more biochemical markers of severity, one of which was a digestive enzyme the other an inflammatory marker. Although 62% publications reported blinded assessment of histological markers of severity, only 19% used two independent assessors and 31% of the studies commented on the effects of an agent on an organ other than pancreas.

Table 5 also summarises the quality assessment of treatments tested in EAP within each category. There were only thirty studies which had a score of > 8 (Warzecha, Dembinski et al. 1999, Mayer, Laine et al. 2000, Gloor, Uhl et al. 2001, Paszkowski, Rau et al. 2002, Alhan, Kalyoncu et al. 2004, Mumcu, Alhan et al. 2005, Yamanel, Mas et al. 2005, Alhan, Turkyilmaz et al. 2006, Alsfasser, Warshaw et al. 2006, Machado, Coelho et al. 2006, Turkyilmaz, Alhan et al. 2008, Liu, Dou et al. 2010, Warzecha, Ceranowicz et al. 2010, You, Tao et al. 2010, Ceyhan, Timm et al. 2011, Paterniti, Mazzon et al. 2012, Wenhong, Jia et al. 2012, Cao and Liu 2013, Nuhn, Mitkus et al. 2013, Huang and Cao 2014, Alhan, Usta et al. 2015, Bukowczan, Warzecha et al. 2015, Chen, Dai et al. 2015, Huang, Cane et al. 2015, Huang, Cash et al. 2015, Mukherjee, Mareninova et al. 2015, Wang, Zeng et al. 2015, Wen, Voronina et

al. 2015, Bukowczan, Cieszkowski et al. 2016, Liu, Zhou et al. 2016). Of all the studies included in the analysis, there were 66 publications which fulfilled the aforementioned clinically relevant criteria, and these have been highlighted in tables 8-14. Further analysis of these publications identified fourteen agents – benzamide (Yasar, Uysal et al. 2010) , IL 10 (Rongione, Kusske et al. 1997) , thymosin alpha 1 (Wang, Zeng et al. 2015), Duchengqi Decoction (Wang, Chen et al. 2012) , ulinastatin (Maciejewski, Burdan et al. 2005) , caffeic acid phenethyl ester (Buyukberber, Savas et al. 2009) , ozone (Uysal, Yasar et al. 2010) , activated protein C (Babu, Genovese et al. 2012) , pentoxifylline (Matheus, Coelho et al. 2009), GSK7975A (Wen, Voronina et al. 2015), DEB025 and TRO40303 (Mukherjee, Mareninova et al. 2015), Obestatin (Bukowczan, Cieszkowski et al. 2016) and polyenoylphosphatidylcholine (Li, Wu et al. 2015) - which showed a greater than 50% improvement in biochemical and histological parameters of severity of EAP.

Table 6 - Quality assessment score and reporting of each parameter

	PARAMETER	SCORE	% studies reporting each parameter
1	***Adequate number of animals per group (6 or more) ****	0 or 1	90
2	Reference to data that inform dose or > 2 doses tested	0 or 1	45
3	***Clinically representative model****	0 or 1	78
4	2 or more models tested	0 or 1	13
5	***Therapeutic administration of treatment****	0 or 1	43
6	Description of approved animal husbandry including board / home office or other standards	0 or 1	84
7	Biochemical blood markers; 2 or more, one of which is digestive enzyme another of which is an inflammatory marker	0 or 1	52
8	***Blinded histopathology score (pancreas) ****	0 or 1	62
9	Two independent blinded assessors of histology score	0 or 1	19
10	Other organ evaluation by biochemical or blinded histological means	0 or 1	31

* ***Highlighted parameters comprised the modified score***

3.3.5 Translation into clinical trials

Table 7 shows the number of agents that have been tested in pre-clinical studies and highlights the ones which have been tested for the treatment of human AP. Although the majority of agents have been evaluated in randomised controlled trials in humans, there are examples of treatment strategies such as hypothermia (da Silva and McWilliams 2013) and use of hyperbaric oxygen (Christophi, Millar et al. 2007) which have been tested in a small number of patients. In summary 282 agents that have been tested pre-clinically as potential treatments for AP and 42 of these have been evaluated for treatment of human AP. Compounds such as oxyphenonium, clonidine, pirenzepine, atropine, valdecoxib and GTN (Cameron, Mehigan et al. 1979, Essardas Daryanani, Santolaria Fernandez et al. 1983, Moreno-Otero, Rodriguez et al. 1989, Lechin, Van der Dijs et al. 1992, Bhatia, Ahuja et al. 2011) have been used for the treatment of human AP without pre-clinical evaluation, although agents of similar category have been tested in experimental models of AP.

Table 7 - Pre-clinical EAP treatment; translation into clinical studies

Treatment	Number of pre-clinical studies	# of agents tested in pre-clinical studies	Agents tested for treatment of human acute pancreatitis
Anti-inflammatory	172	123	Methyl prednisolone (Dumot, Conwell et al. 1998), Dexamethasone(Wang, Liu et al. 2004), Hydrocortisone (De Palma and Catanzano 1999, Manolakopoulos, Avgerinos et al. 2002, Kwanngern, Tiyapattanaputi et al. 2005), IL 10 (Deviere, Le Moine et al. 2001, Dumot, Conwell et al. 2001), Lexipafant (Kingsnorth, Galloway et al. 1995, McKay, Curran et al. 1997, Johnson, Kingsnorth et al. 2001), 5 FU (Tonetti and Calvi 1989), Thymosin alpha 1 (Wang, Li et al. 2011), Aprepitant (Shah, Liddle et al. 2012) , Salvia miltiorrhizae (Wang, Wen et al. 2013), Duchengqi decoction (Zhang, Zhang et al. 2008, Wan, Li et al. 2011), Acetylhydrolase (Sherman, Alazmi et al. 2009) and ***hypothermia* (da Silva and McWilliams 2013)***
Enzyme inhibitors	28	21	Gabexate (Andriulli, Solmi et al. 2004, Manes, Ardizzone et al. 2007, Pezzilli, Uomo et al. 2007, Ino, Arita et al. 2008), Nafamostat (Choi, Kang et al. 2009, Piascik, Rydzewska et al. 2010, Yoo, Huh et al. 2011, Park, Jeon et al. 2014), Urinastatin (Yoo, Ryu et al. 2008, Itaba, Nakamura et al. 2013, Park, Jeon et al. 2014, Wang, Su et al. 2014), Aprotinin (Pederzoli, Cavallini et al. 1993, Berling, Genell et al. 1994), Magnesium (Fluhr, Mayerle et al. 2013), FFP (Leese, Holliday et al. 1987, Leese, Holliday et al. 1991) and Camostat (Freise, Schmidt et al. 1985)

Anti-oxidants	52	32	N-acetylcysteine (Katsinelos, Kountouras et al. 2005, Milewski, Rydzewska et al. 2006, Siriwardena, Mason et al. 2007), Selenium (Virlos, Mason et al. 2003, Siriwardena, Mason et al. 2007) , Allopurinol (Budzynska, Marek et al. 2001, Katsinelos, Kountouras et al. 2005, Romagnuolo, Hilsden et al. 2008, Martinez-Torres, Rodriguez-Lomeli et al. 2009), Vitamins A,B, C ,E (Du, Yuan et al. 2003, Bansal, Bhalla et al. 2011), ***Hyperbaric oxygen**** (Christophi, Millar et al. 2007) and ***β-carotene†(Lavy, Karban et al. 2004)***
Secretion inhibitors	40	20	Octreotide (Li, Pan et al. 2007, Yang, Wu et al. 2012, Wang, Yang et al. 2013), ***Loxiglumide* (Ochi, Harada et al. 1999),*** Sandostatin (Saenko, Lupal'tsov et al. 1999), Secretin (Jowell, Branch et al. 2011), Cimetidine (Loiudice, Lang et al. 1984, Navarro, Ros et al. 1984), Glucagon (Debas, Hancock et al. 1980, Kronborg, Bulow et al. 1980, Regan, Malagelada et al. 1981), ***Clonidine*† (Lechin, Van der Dijs et al. 1992), Pirenzepine†, (Moreno-Otero, Rodriguez et al. 1989), Oxyphenonium†(Essardas Daryanani, Santolaria Fernandez et al. 1983) and Atropine†(Cameron, Mehigan et al. 1979)***
Microcirculat-ion and blood flow	53	38	Heparin (Berger, Quera et al. 2001) , LMWH (Rabenstein, Fischer et al. 2004, Barkay, Niv et al. 2008, Lu, Qiu et al. 2009), Activated protein C (Pettila, Kyhala et al. 2010),**human recombinant Activated protein C** * ***(Miranda, Mason et al. 2015***), Thyrotropin releasing hormone (Kiviniemi, Laitinen et al. 1986) and ***Dextran* (Klar, Foitzik et al. 1993, Wang, Liu et al. 2004)***
Eicosanoids	24	23	Indomethacin (Elmunzer, Scheiman et al. 2012, Dobronte, Szepes et al. 2014), Omega 3 fatty acids (Lasztity, Hamvas et

			al. 2005), Diclofenac† (Otsuka, Kawazoe et al. 2012, Park, Chung et al. 2015), ***Valdecoxib†(Bhatia, Ahuja et al. 2011)*** and CaNa2EDTA (Tykka, Vaittinen et al. 1985)
Others	61	25	***GTN† (Bhatia, Ahuja et al. 2011)***, peritoneal dialysis(Yang, Guanghua et al. 2010), Nifedipine (Prat, Amaris et al. 2002), Insulin (Svensson 1975), Calcitonin (Martinez and Navarrete 1984) and Pentoxifylline (Kapetanos, Christodoulou et al. 2009, Vege, Atwal et al. 2015)

**** Compounds tested in non-randomised clinical trials, others evaluated in RCT's***

† Compounds tested directly in clinical trials without being tested in EAP

Table 8 - Anti-inflammatory agents tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	16979620	3-aminobenzamide	Mazzon	2006	CER-AP	Prophylactic	Improved	Improved
2	16127429	3-aminobenzamide	Mota	2005	CER-AP	Prophylactic	Improved	Improved
3	19399939	5 Fluorouracil	Cheng	2009	TLCS-AP	Therapeutic	Mild effect	Not reported
4	21400110	a,ß-amyrin	Melo	2001	CER-AP	Prophylactic	Improved	Improved
5	20818810	a,ß-amyrin	Melo	2010	L – arginine	Therapeutic	Improved	Improved
6	20531239	Adalimumab	Yilmaz	2010	TLCS-AP	Prophylactic	Improved	Improved
7	23625750	Adenosine A3 receptor agonist – IB-MECA	Prozorow-Krol	2013	TLCS-AP	Prophylactic	Mild effect	Mild effect
8	***17928102***	***Adrenomedullin***	***Onur***	***2007***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***
9	8540653	Anti TNF α polyclonal antibody	Hughes	1996	Retrograde infusion of bile acid + trypsin	Prophylactic	Mild effect	Improved
10	8311136	Anti TNF α polyclonal antibody	Grewal	1994	Retrograde infusion of bile acid + trypsin	Prophylactic	Improved	No effect
11	25895924	Anti-Gr-1 antibody	Cheng	2015	L - Arginine	Prophylactic	Improved	Improved
12	17014919	Antileukinate	Bhatia	2007	CER-AP	Prophylactic	Improved	Improved
13	23918150	Apamin - bee venom derivative	Bae	2013	CER-AP	Prophylactic	Improved	Improved
14	12643851	Batimistat	Muhs	2003	TLCS-AP	Prophylactic	Improved	Improved
15	21939783	Bee venom	Yun Sw	2011	CER-AP	Prophylactic	Improved	Improved
16	18376296	Bee venom	Seo	2008	CER-AP	Prophylactic	Improved	Improved
17	***20705631***	***Benzamide***	***Yasar***	***2010***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
18	20382594	Bindarit	Zhout	2010	TLCS-AP	Prophylactic	Improved	Improved
19	15691869	Bindarit	Bhatia	2005	CER-AP	Prophylactic	Mild effect	Improved
20	19486901	Bortezomib	Szabolcs	2009	CER-AP	Prophylactic	No effect	Mild effect
21	21528054	Breviscapine	Zhang	2011	Bile acid induced	Therapeutic	Improved	Improved

22	8370981	C1 - choline-esterase inhibitor	Vesentini	1993	TLCS-AP	Prophylactic	Not reported	No effect
23	7622941	C1 esterase inhibitor	Niederau	1995	CER-AP, TLCS-AP and CDE diet	Prophylactic	No effect	No effect
24	22626927	Caesalpinia pyramidalis extract	Santanu	2012	Bile duct ligation	Prophylactic	Improved	Not reported
25	15168010	Calpain I inhibitor	Virlos	2004	CER-AP	Prophylactic	Improved	Improved
26	14625479	Calpain I inhibitor - Pyrrolidine dithiocarbamate	Virlos	2003	CER-AP	Prophylactic	Improved	Improved
27	19997973	Calpain I inhibitor- Pyrrolidine dithiocarbamate	Zhang	2010	TLCS-AP	Prophylactic	Improved	Improved
28	25287011	Calpain I inhibitor- Pyrrolidine dithiocarbamate	Xu	2015	TLCS-AP	Prophylactic	Improved	Improved
29	22850623	Cannabidiol & 01607	Li	2013	CER-AP	Prophylactic	Improved	Improved
30	17484889	Cannabinoids	Michalski	2007	CER-AP	Prophylactic	Improved	Improved
31	19629004	Capsaicin	Schneider	2009	Bile acid and caerulein infusion	Prophylactic	Improved	Improved
32	16707851	Captopril	Cheng	2006	TLCS-AP	Prophylactic	Improved	Improved
33	24334457	CO-releasing molecule–2	Xue	2014	CER-AP and CDE diet	Prophylactic	Improved	Improved
34	22199113	Curcuma longo (tumeric)	Yu Wg	2011	CER-AP	Prophylactic	Improved	Not reported
35	***23000891***	***Desferrioxamine, Biliverdin and Methylene Chloride***	***Nuhn***	***2013***	***TLCS-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
36	17625291	Dexamethasone	Zhang	2007	TLCS-AP	Therapeutic	Not reported	Improved
37	19050604	Dexamethasone	Yubero	2009	TLCS-AP	Prophylactic & Therapeutic	Improved	Not reported
38	***26770346***	***Diclofenac***	***Ozer***	***2015***	***CER-AP***	***Therapeutic***	***No effect***	***Improved***
39	24966921	Diosmentin	Yu	2014	CER-AP	Prophylactic	Improved	Improved
40	***22768339***	***Duchengqi Decoction***	***Wang***	***2012***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
41	17653597	Enalaprilat	Turkyilmaz	2007	CER-AP	Therapeutic	Improved	Improved
42	19840765	Etanercept	Yilmaz	2009	TLCS-AP	Prophylactic	Improved	Improved
43	17438460	Etanercept	Malleo	2008	CER-AP	Prophylactic	Improved	Improved
44	18680237	Ethyl pyruvate	Yang	2008	TLCS-AP	Therapeutic	Improved	Improved

45	17895847	Ethyl pyruvate	Cheng	2007	TLCS-AP	Prophylactic	Improved	Not reported
46	***23600830***	***Ethyl pyruvate***	***Luan***	***2013***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
47	***22717227***	***Ethyl Pyruvate***	***Luan***	***2013***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
48	2370242	Eucalyptol	Lima	2013	CER-AP	Prophylactic	Improved	Improved
49	***26668524***	***Everolimus***	***Özkardeş***	***2015***	***CER-AP***	***Therapeutic***	***No effect***	***No effect***
50	24177139	Fucoidan	Carvalho	2014	CER-AP and TLCS-AP	Prophylactic	Improved	Improved
51	25479110	G5 PAMAM dendrimers	Tang	2015	CER-AP	Prophylactic	improved	improved
52	18985809	Gardenia jasminoides	Jung	2008	CER-AP	Prophylactic	Improved	Improved
53	***15378786***	***Gene therapy - Human IL-10 gene***	***Chen***	***2004***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
54	25759121	Gentiopicroside	Lv	2015	TLCS-AP	Prophylactic	improved	improved
55	21474970	Glycine	Ceyhan	2011	CER-AP and TLCS-AP	Prophylactic	Improved	Mild effect
56	18074481	GSK-3beta inhibitor - TDZD 8	Cuzzocrea	2007	CER-AP	Prophylactic	Improved	Improved
57	25843255	Guggulsterone	Kim	2015	CER-AP	Prophylactic	Improved	Improved
58	18486906	Human leukocyte elastase inhibitor	Jo	2008	CER-AP	Prophylactic	Improved	Improved
59	***11680590***	***Hydrocortisone***	***Gloor***	***2001***	***CER-AP and TLCS - AP***	***Therapeutic***	***Improved***	***No effect***
60	***17406762***	***Hyperthermia***	***Almeida***	***2006***	***CER-AP***	***Therapeutic***	***No effect***	***Mild effect***
61	***11753044***	***IL 1 beta converting enzyme inhibitor***	***Paszkowski***	***2002***	***TLCS - AP***	***Therapeutic***	***Improved***	***Mild effect***
62	7539389	IL-10	Van Laethem	1995	CER-AP	Prophylactic	Improved	Improved
63	15804946	IL-10	Keceli	2005	CER-AP	Prophylactic	Improved	Improved
64	***9041259***	***IL-10***	***Rongioneet***	***1997***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
65	8751594	IL-10	Kusske	1996	CDE diet induced	Prophylactic & Therapeutic	Improved	Improved
66	7539942	IL-1ra	Norman	1995	CER-AP	Prophylactic	Improved	Improved

67	7794067	IL-1ra	Norman	1995	CDE diet	Prophylactic & Therapeutic	Improved	Improved
68	7736749	IL-1ra	Tanaka	1995	TLCS-AP	Prophylactic	Improved	Improved
69	26026064	IL-6 antagonist - LMT-28	Hing	2015	CER-AP	Prophylactic & Therapeutic	Improved	Improved
70	10189850	IL-8 antibody	Osman	1998	Bile acid + PD ligation	Prophylactic	Improved	Mild effect
71	22982818	Inflexinol	Ahn	2013	CER-AP	Prophylactic	Improved	Mild effect
72	14707742	Infliximab	Oruc	2004	CER-AP and TLCS-AP	Prophylactic	Improved	Improved
73	25869565	Infliximab	Tekin	2015	TLCS-AP	Prophylactic	Improved	Improved
74	16531145	Inosine	Schneider	2006	Bile acid followed by caerulein	Prophylactic & Therapeutic	Not reported	Improved
75	14767733	Inosine	Yamagiwa	2004	CER-AP	Prophylactic	Improved	Mild effect
76	***17513789***	***Interferon gamma***	***Hayashi***	***2007***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
77	21369693	JAK/STAT inhibitors - Rapamycin & AG490	Cheng	2011	CER-AP	Prophylactic	Improved	Improved
78	15454338	JNK inhibitor - SP600125	Minutoli	2004	CER-AP	Prophylactic	Improved	Improved
79	19770782	Leflunomide	Kutluana	2010	TLCS-AP	Prophylactic	Mild effect	Improved
80	12510863	Leptin	Warzecha	2002	Ischemia-induced	Therapeutic	time course measurements	time course measurements
81	11469912	Lexipafant	Lane	2001	CER-AP	Prophylactic	Improved	No effect
82	9629507	Lexipafant	Rivera	1998	Bile acid + CER and Enterokinase + CER	Therapeutic	Not reported	No effect
83	***25265022***	***Lipoxin A4***	***Yang***	***2014***	***Bile acid infusion***	***Therapeutic***	***Improved***	***Improved***
84	25102438	Lithospermum erythrorhizon	Choi	2015	CER-AP	Prophylactic	Improved	Improved
85	26407655	Loganin	Kim	2015	CER-AP	Prophylactic & therapeutic	Improved	Improved
86	14643897	Losartan and PD 123319	Tsang	2004	CER-AP	Prophylactic & Therapeutic	Mild effect	Mild effect
87	16079481	Lovastatin	Choi	2005	CER AP	Prophylactic	Improved	Not reported
88	26179197	Lupeol	Kim	2015	CER-AP	Prophylactic	Improved	Improved

89	12370536	MAP kinase inhibitors PD98059 & U0126	Clemons	2002	CER-AP	Prophylactic	Improved	Improved
90	12484556	Methimazole	Yonetci	2002	CER-AP	Prophylactic	Improved	Improved
91	12162412	Methyl prednisolone	Takaoka	2002	Closed duodenal loop	Prophylactic	Improved	Improved
92	18437083	Methyl prednisolone	Paszt	2008	L-arginine-induced	Prophylactic	Improved	Improved
93	***15841032***	***Mild hypothermia***	***Wang***	***2005***	***TLCS - AP***	***Therapeutic***	***Improved***	***Mild effect***
94	20467345	Montelukast	Ozkan	2010	CER-AP	Prophylactic	Improved	Not reported
95	20924190	Mycophenolate mofetil	Tasdogan	2010	CER-AP	Therapeutic	Improved	Improved
96	25914464	Na HS (H2S donor)	Rao	2015	L- Arginine	Prophylactic	Improved	Improved
97	19940795	Nardostachys jatamanis	Bae	2010	CER-AP	Prophylactic	Improved	Improved
98	22783046	Nardostachys jatamanis extract	Bae	2012	CER-AP	Prophylactic	Improved	Improved
99	23029434	Netrin-1	Cheng	2013	L-arginine	Therapeutic	Improved	Improved
100	25742430	Nicotine	Zheng	2015	TLCS-AP	Prophylactic	Improved	Improved
101	12826913	NK1 receptor antagonist – CP-96345	He	2003	Intraductal infusion of meglumine	Prophylactic	Mild effect	Mild effect
102	22850625	Noggin	Cao	2013	CER-AP	Prophylactic	Improved	Improved
103	25931351	Oleuropein	Caglayan	2015	Bile-pancreatic duct obstruction	Prophylactic	No effect	No effect
104	24326366	Opuntia humifusa	Choi	2014	CER-AP	Prophylactic	Improved	Improved
105	***11034471***	***Orthoclone & Tacrolimus***	***Mayer***	***2000***	***CER-AP***	***Therapeutic***	***Mild effect***	***Improved***
106	17895833	Oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OXPAPC)	Li	2007	CER-AP and TLCS-AP	Prophylactic & Therapeutic	Improved	Improved
107	21633783	Oxymatrine	Zhang	2012	L-Argnine	Therapeutic	Improved	Not reported
108	25655298	p38 MAPK inhibitor - SB203580	Cao	2015	CER-AP	Prophylactic	Improved	Improved
109	16534854	Patrinia scabiosaefolia	Seo	2006	CER-AP	Prophylactic	Improved	Not reported
110	23275617	Pepducin	Michael	2012	CER-AP and TLCS-AP	Prophylactic	Mild effect	Mild effect

111	25950520	Pepducin or anti-Ly6G	Steele	2015	CER-AP	Prophylactic & Therapeutic	Improved	Improved
112	6526613	Phenylbutazone	Louagie	1984	Intraductal injection of trypsin	Prophylactic	No effect	Improved
113	22677361	Pioglitazone	Wan	2012	CER-AP	Prophylactic	Improved	Improved
114	21663734	Piperine	Bae	2011	CER - AP	Prophylactic	Improved	Improved
115	7827939	Platelet-activating factor antagonist - BB-882	Formela	1994	Microvascular ischaemia	Therapeutic	Improved	Improved
116	1294265	Platelet-activating factor antagonist - TCV-309	Tomaszewska	1992	CER-AP	Prophylactic	Improved	Improved
117	1571504	Platelet-activating factor antagonists - CV-6209	Fujimura	1992	CER-AP	Prophylactic	Improved	Improved
118	7537159	Platelet-activating factor antagonists BN-52021 & WEB-2170	Jancar	1995	CER-AP	Prophylactic	Improved	Not reported
119	26302488	Polysialic acide & DNAse1	Merza	2015	TLCs-AP and L-arginine	Prophylactic	Improved	Improved
120	22722259	PPAR α agonist - WY14643	Ding	2013	CER-AP	Prophylactic	Improved	Improved
121	22521259	PPAR β/δ agonist - GW0742	Paternitis	2012	CER-AP and TLCS-AP	Prophylactic & Therapeutic	Improved	Improved
122	***16966031***	***PPAR-gamma agonists***	***Rollins***	***2006***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
123	***19530045***	***Propolis***	***Büyükberber***	***2009***	***CER-AP***	***Prophylactic & Therapeutic***	***Mild effect***	***Mild effect***
124	17724800	Protasome inhibitor - MG132	Letoha	2007	CER-AP	Prophylactic	Improved	Improved
125	16214030	Protasome inhibitor - MG132	Letoha	2005	CER-AP	Prophylactic	Improved	Improved
126	17347056	Protease and PKC inhibitors - aprotinin, pefabloc, trypsin inhibitor & polymyxin B, staurosporine	Shi	2007	Bile acid induced	Prophylactic	Improved	Not reported
127	10360224	Protease inhibitors :C1-esterase inhibitor, α-2 anti-plasmin, antithrombin III,α -1-macroglobulin and α-1-	Kruse	1999	TLCS-AP	Prophylactic	Mild effect	No effect

		protease inhibitor						
128	20006347	Proteasome Inhibitor - PS-341	Dong	2010	Caerulein + LPS	Prophylactic	Improved	Improved
129	22481287	Rapamycin, cortisol & FTY720	Muller	2012	TLCS-AP	Therapeutic	Mild effect	Mild effect
130	25460024	Ras inhibitor - farnesylthiosalicylic acid	Yu	2015	TLCS-AP	Prophylactic	Improved	Improved
131	20600562	Recombinant fibrinogenase II	Luo	2010	TLCS-AP	Therapeutic	Improved	Not reported
132	10975706	Recombinant human IL-11	Shimizu	2000	Caerulein + LPS	Prophylactic	Improved	Mild effect
133	26702138	Resolvin D1	Liu	2015	CER-AP & LPS	Prophylactic	Improved	Improved
134	15641139	Resveratrol	Meng	2005	TLCS-AP	Therapeutic	Improved	Improved
135	***23240749***	***Rhubarb***	***Feng***	***2012***	***TLCS - AP***	***Therapeutic***	***Mild effect***	***Mild effect***
136	15052683	Rhubarb	Zhao	2004	CER +5 hr restraint water immersion stress	Prophylactic	Improved	Improved
137	19117125	Rolipram	Mersin	2009	TLCS-AP	Prophylactic	Improved	Improved
138	16759642	Rolipram	Sato	2010	CER-AP	Prophylactic	Improved	Improved
139	14985957	Rosiglitazone	Cuzzocrea	2004	CER-AP	Prophylactic	Improved	Improved
140	17072978	Rosiglitazone	Folch-Puy	2006	Intraductal contrast	Prophylactic	Improved	Improved
141	20408879	Rosiglitazone	Sindhu	2011	L-Arginine and L-Arginine followed by CCK	Therapeutic	Improved	Improved
142	19114961	Rosiglitazone	Celinski	2009	TLCS-AP	Prophylactic	Improved	Improved
143	19238530	Salvia miltiorrhizae	Zhang	2009	TLCS-AP	Therapeutic	Mild effect	Improved
144	12657946	Saralasin	Ips	2003	CER-AP	Prophylactic	Improved	Improved
145	12609748	Saralasin & Ramiprilat	Tsang	2003	CER-AP	Prophylactic	Mild effect	Mild effect
146	***24738018***	***Shen Fu***	***Huang***	***2014***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***
147	23201088	Shiknoin	Xiong	2013	CER-AP	Prophylactic	Improved	Improved
148	18648134	Simvastatin	Almeida	2008	CER - AP and TLCS-AP	Prophylactic	Mild effect	No effect
149	***23794035***	***Sivelestat***	***Cao***	***2013***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***

150	25686746	Sodium butyrate	Zhang	2015	L - Arginine	Therapeutic	Improved	Improved
151	25948069	Sphingosine-1-phosphate type-1 receptor agonist - SWE2871	Zou	2015	CER-AP	Prophylactic	Improved	Improved
152	22396778	S-propargyl-cysteine	Sidhapuriwala	2012	CER-AP	Prophylactic	Improved	Not reported
153	18815555	Synacthen and cortisol	Muller	2008	TLCS-AP	Therapeutic	Mild effect	Mild effect
154	***20402659***	***Tacrolimus***	***Liu***	***2010***	***TLCS-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
155	15641154	Taraxacum officinale	Seo	2005	CER-AP	Prophylactic	Improved	Not reported
156	26557854	Tetrandrine	Wu	2015	TLCS-AP	Therapeutic	Improved	Improved
157	18246603	Thalidomide	Malleo	2008	CER-AP	Prophylactic	Improved	Improved
158	17295769	Thalidomide	Xiong	2007	Intraductal contrast	Prophylactic	Improved	Improved
159	21437599	Thalidomide analog - Pomalidomide	Tsai	2011	CER-AP	Prophylactic & Therapeutic	Improved	Improved
160	***17914961***	***Thymosin α 1***	***Yao***	***2007***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***Improved***
161	***26330363***	***Thymosin α1 and interferon α***	***Wang***	***2015***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
162	26668646	Thymosin β4	He	2015	TLCS-AP	Prophylactic	Improved	Improved
163	25492506	Trichostatin	Hartman	2015	TLCS-AP	Prophylactic	Improved	Improved
164	18308855	Tripeptide analog - feG	Rifai	2008	CER-AP	Prophylactic & Therapeutic	Improved	Improved
165	21778460	Trismethoxymethoxy chalcone (TMMC)	Kim	2011	CER-AP	Prophylactic	Improved	Improved
166	21778460	TMMC	Kin	2011	CER-AP	Prophylactic	Improved	Improved
167	12666696	Tyrosine kinas inhibitor - Tyrphostin AG 556	Alhan	2002	CER + bile acid	Therapeutic	Improved	Improved
168	11044526	Whole gut washout	Yol	2004	Pancreatic duct ligation	Therapeutic	Improved	Improved
169	22529518	Zerumbone	Wenhong	2012	TLCS - AP	Prophylactic	Improved	Improved
170	17895846	Zerumbone	Szabolcs	2007	CER-AP	Prophylactic	Improved	No effect

171	15003365	α-melanocyte stimulating hormone	Jahovic	2004	CER-AP	Prophylactic	Improved	Improved
172	22982349	α-pinene	Bae	2012	CER-AP	Prophylactic	Improved	Improved

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 9 - Enzyme inhibitors tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	313758	Aprotinin and chlorophyll a	Manabe	1989	CDE diet	Prophylactic	No effect	No effect
2	6199589	Aprotinin and Dextran 40	Crocket	1984	Closed-duodenal-loop	Prophylactic	Improved	Improved
3	***1689237***	***Camostat (FOY-305)***	***Otsuki***	***1990***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***
4	12181196	Cathepsin B inhibitor	Van Acker	2002	CER-AP and TLCS-AP	Prophylactic	Improved	Improved
5	9094156	Chloroquine	Guillaumes	1997	CDE diet	Prophylactic	Improved	Not reported
6	8500736	Chloroquine	Lerch	1993	CER - AP and CDE diet	Prophylactic	No effect	No effect
7	20448143	Dantrolene	Orabi	2010	Caerulein + LPS	Prophylactic	Improved	Improved
8	8071570	E3123 (synthetic protease inhibitor)	Sata	1994	CER-AP	Prophylactic & Therapeutic	Mild effect	Improved
9	1383065	E-3123 (synthetic protease inhibitor)	Hirano	1992	CER-AP	Prophylactic	Improved	Improved
10	7826181	Gabexate mesilate	Hirano	1994	i.v ethanol infusion	Prophylactic	Improved	Improved
11	1717033	Gabexate mesilate	Hirano	1991	CER-AP	Prophylactic	Improved	Improved
12	2442741	Gabexate mesilate	Wisner	1987	CER-AP	Prophylactic	Improved	Improved
13	2414198	Glutaryl-trialanine-ethylamide	Fric	1985	TLCS-AP	Prophylactic & Therapeutic	Improved	Improved
14	10206480	Guamerin-derived synthetic peptide	Song	1999	CER-AP	Prophylactic	Improved	Improved
15	1459347	Human pancreatic secretory trypsin inhibitor	Funakoshi	1992	CER-AP	Prophylactic	Improved	Improved
16	6363018	Human urinary trypsin inhibitor - MTI	Ohnishi	1984	Closed duodenal loop and trypsin + bile acid	Prophylactic	Improved	Not reported
17	6182054	Leupeptin	Jones	1982	Bile acid + trypsin	Therapeutic	Not reported	Not reported
18	24277728	Magnesium	Schick	2014	CER-AP	Prophylactic	Improved	Improved

19	18362838	Nafamostat	Lee	2008	CER-AP	Prophylactic	Improved	Improved
20	15782103	Nafamostat	Mikami	2005	TLCS-AP	Therapeutic	Improved	Improved
21	2732529	Nafamostat	Wisner	1989	CER-AP	Prophylactic	Improved	Improved
22	1381836	ONO3307 & FOY007	Hirano	1992	CER-AP	Prophylactic	Improved	Improved
23	10467964	Sepimostat	Yuasa	1999	CER-AP, Caerulein + ethanol and ethanol induced	Prophylactic	Improved	Mild effect
24	9717766	Trifluoroacetyl-L-lysyl-L-alaninanilide hydrochloride	Yamano M	1998	CER-AP, closed duodenal loop and bile acid + trypsin infusion	Prophylactic	Improved	Improved
25	1374785	Trypsin inhibitor - FUT-175	Suzuki	1992	CDE diet	Prophylactic & Therapeutic	Mild effect	No effect
26	***15816359***	***Ulinastatin***	***Maciejewski***	***2005***	***TLCS-AP***	***Therapeutic***	***Improved***	***Mild effect***
27	2459695	Urinastatin	Tani	1988	CER-AP	Prophylactic	Mild effect	Improved
28	7681748	Urinastatin	Hirano	1993	CER-AP	Prophylactic	Improved	Improved

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 10 - Antioxidants tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	8530831	4-hydroxy-TEMPO (superoxide dismutase mimic)	Sledzinski	1995	CER-AP	Prophylactic	Not reported	Improved
2	19371263	Acetyl-L-carnitine	Arafa	2009	CER-AP	Prophylactic	Improved	Improved
3	***17287560***	***Allopurinol***	***Isik***	***2006***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
4	2762273	Allopurinol	Lankisch	1989	TLCS-AP and CDE diet induced	Prophylactic	No effect	No effect
5	2456257	Allopurinol	Wisner	1988	CER-AP	Prophylactic	Improved	Not reported
6	16097064	Alpha-lipoic acid	Park	2005	CER-AP	Prophylactic	Mild effect	Improved
7	9700946	Artemisia asiatica extract - DA 9601	Hahm	1998	CER-AP	Prophylactic	No effect	Mild effect
8	***19891017***	***Caffeic acid phenethyl ester***	**Buyukberber**	***2009***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
9	18028950	Caffeic acid phenyl Ester	Turkyilmaz	2008	CER-AP	Therapeutic	Mild effect	Improved
10	26093481	Carvacrol	Kılıç	2016	CER-AP	Therapeutic	Improved	Improved
11	15028960	Chondroitin sulphate	Campo	2004	CER-AP	Prophylactic	Improved	Not reported
12	18408139	Chondroitin sulphate	Campo	2008	CER-AP	Prophylactic	Improved	Not reported
13	1723549	Dimethylsulfoxide-hydroxyl	Dabrowski	1991	CER-AP	Prophylactic	Mild effect	No effect
14	12792821	Edaravone	Araki	2003	Closed duodenal loop	Prophylactic	Improved	Improved
15	1370933	Free radical scavenger - CV3611	Nonaka	1992	CER-AP	Prophylactic	Improved	Improved
16	1710198	Free radical scavenger - CV3611	Nonaka	1991	CDE diet induced	Prophylactic & Therapeutic	Improved	Not reported
17	14697296	Gingko biloba extract - EGb 761	Zeybek	2003	TLCS-AP	Prophylactic	Improved	No effect
18	15613745	Grapefruit seed extract	Dembinski	2004	Ischemia-reperfusion	Prophylactic	Improved	Not reported
19	7479668	Green tea catechins -	Takabayasi	1995	Ethionine induced	Prophylactic	Improved	Not reported

		Polyphenon)						
20	19672210	Green tea polyphenol	Babu	2009	CER-AP	Prophylactic	Improved	Improved
21	22695421	Hydrogen rich saline	Ren	2012	Traumatic pancreatitis	Therapeutic	Mild effect	No effect
22	20138831	Hydrogen rich saline	Chen	2010	L-arginine	Therapeutic	Improved	Not reported
23	25214720	Hydrogen rich saline	Ren	2014	CER-AP	Therapeutic	Improved	Mild effect
24	19174608	Hyperbaric oxygen	Yu	2009	TLCS-AP	Therapeutic	Improved	Improved
25	***17623267***	***Hyperbaric oxygen***	***Nikfarjam***	***2007***	***TLCS-AP***	***Therapeutic***	***No effect***	***Improved***
26	***18469439***	***Hyperbaric oxygen***	***Festugato***	***2008***	***Pancreatic duct ligation***	***Therapeutic***	***Not reported***	***No effect***
27	***19823101***	***Hyperbaric oxygen & ozone***	***Uysal***	***2010***	***TLCS-AP***	***Therapeutic***	***Improved***	***Mild effect***
28	16525355	Hypericum perforatum	Genovese	2006	CER-AP	Prophylactic	Improved	Improved
29	7686520	Lactoferrin	Koike	1993	CER-AP	Prophylactic	Improved	Improved
30	***11373483***	***Lazaroid***	***Schulz***	***2001***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***No effect***
31	16557023	Lazaroid	Alhan	2006	CER-AP	Therapeutic	Mild effect	No effect
32	***25878403***	***Mitoquinone***	***Huang***	***2015***	***CER-AP and TLCS-AP***	***Prophylactic & Therapeutic***	***Effective in CER-AP only***	***Effective in CER-AP only***
33	***16088183***	***N-acetylcysteine***	***Mumcu***	***2005***	***Caerulein + bile acid***	***Therapeutic***	***Improved***	***Mild effect***
34	15065005	N-acetylcysteine	Yagci	2004	TLCS-AP	Prophylactic	Mild effect	Improved
35	12949437	N-acetylcysteine	Sevillano	2003	Pancreatic duct obstruction	Prophylactic	Improved	Not reported
36	10707932	N-acetylcysteine	Demols	2000	CER-AP and CDE diet	Prophylactic	Improved	Mild effect
37	10707932	N-acetylcysteine	Demols	2000	CER-AP and CDE diet	Prophylactic	Improved	Improved
38	19050604	N-acetylcysteine & dexamethasone	Yubero	2009	TLCS-AP	Prophylactic	Improved	Not reported
39	12747737	Raxotelast	Altavilla	2003	CER-AP	Prophylactic	Improved	Improved

40	16499907	Resveratrol	Szabolcs	2006	CER-AP	Prophylactic	Mild effect	Mild effect
41	16186664	Selenium	Hardman	2005	L-arginine	Therapeutic	No effect	Improved
42	15980666	Selenium, ascorbate & N-acetylcysteine	Hardman	2005	L-arginine	Therapeutic	Mild effect	Mild effect
43	22076497	Sesamol	Chu	2012	CER-AP	Prophylactic	Improved	Improved
44	8174416	Superoxide dismutase	Schoenberg	1994	TLCS-AP	Prophylactic	Improved	Mild effect
45	15316396	Superoxide dismutase mimetic - M40401	Cuzzocrea	2004	CER-AP	Prophylactic	Improved	Improved
46	26011211	Syringic acid	Cikman	2015	L-Arginine	Therapeutic	Not reported	Improved
47	11408826	Taurine	Ahn	2001	CER-AP	Prophylactic	Mild effect	Improved
48	***23536517***	***Taurine***	**Akay**	***2013***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***No effect***
49	26770650	Tempol	Marciniak	2016	CER-AP	Prophylactic	Improved	Improved
50	26376346	Tempol	Erbıs	2015	Caerulein + bile acid	Therapeutic	Improved	Improved
51	12667389	Tiopronin	Cui	2003	TLCS-AP	Prophylactic	Improved	Improved
52	23484468	Trimetazidine	Yenicerioglu	2013	L-arginine	Therapeutic	Improved	Improved

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 11 - Secretion Inhibitors tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	2453874	Asperlicin	Wisner	1988	TLCS-AP	Prophylactic	Improved	Not reported
2	2465158	CCK antagonist - L 364,718	Sjovall	1988	TLCS-AP	Prophylactic	No effect	Not reported
3	2813332	CCK antagonist - L364,718	Ohshio	1989	CER-AP and CDE diet	Prophylactic	No effect	No effect
4	9788544	CCK antagonist - TS-941	Wang	1998	CER-AP and intraductal infusion of bile acid + trypsin	Prophylactic	Improved	Not reported
5	15331911	CCK-1 antagonist - IQM-97,423	Latorre	2004	Pancreatic duct ligation and CER-AP	Prophylactic	Improved	Not reported
6	9074944	CCK-A antagonist - T-0632	Taniguchi	1997	CER-AP and pancreatic duct ligation	Prophylactic	Improved	Not reported
7	8975946	CCK-antagonist - CR1409	Niederau	1996	Pancreaticobiliary duct obstruction	Therapeutic	Improved	Improved
8	3104890	CCK-antagonist - CR1409	Makovec	1986	CER-AP and TLCS-AP	Prophylactic	Improved	Not reported
9	7524065	CCK-antagonist - KSG 504	Ha	1994	Closed duodenal loop	**Therapeutic**	Improved	mild effect
10	***2474165***	***CR 1392***	***Otsuki***	***1989***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***no effect***
11	***20664821***	***Diphenhydramine***	***You N***	***2010***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
12	602326	Glucagon	Durr HK	1977	TLCS-AP and pancreatic duct ligation	Prophylactic & Therapeutic	Not reported	Not reported
13	7517544	L-364,718	Garcia-Montero	1994	CER-AP	Prophylactic & Therapeutic	Improved	Improved
14	1696383	L-364,718	Murayama	1990	Gastric, duodenal, bile, pancreatic fistulae and CER-AP	Prophylactic	Improved	not reported

15	***1380426***	***Long-acting somatostatin analogue -***	***van Ooijen***	***1992***	***TLCS-AP***	***Prophylactic & Therapeutic***	***Improved***	***Mild effect***
16	2581647	Long-acting somatostatin analogue - SMS 201-995	Baxter	1985	Pancreatic duct ligation	Prophylactic & Therapeutic	Improved	Not reported
17	2343042	Loxiglumide	Tani	1990	CER-AP and TLCS-AP	Prophylactic	Improved	Improved
18	10026435	Loxiglumide	Satake	1999	CER-AP , TLCS-AP and closed duodenal loop	Prophylactic & Therapeutic	Improved	Not reported
19	8720664	MCI-727 (anti-ulcer properties)	Tachibana	1996	CER-AP and TLCS-AP	Prophylactic & Therapeutic	Improved	Improved
20	23959212	Neuronostatin	Mo	2013	TLCS-AP	Prophylactic	Improved	Improved
21	10095153	Ocreotide	Greenberg	1999	Intra-parenchymal injections of bile acid	Therapeutic	Improved	Improved
22	17914962	Octreotide	Wenger	2007	Bile acid + caerulein	Therapeutic	Improved	Improved
23	12748426	Octreotide	Salem	2003	TLCS-AP	Prophylactic	No effect	No effect
24	10533767	Octreotide	Küçüktülü	1999	CER-AP and TLCS-AP	Prophylactic	Mild effect	Mild effect
25	9873955	Octreotide	Marton	1998	TLCS-AP	Therapeutic	Improved	No effect
26	***9533643***	***Octreotide***	***Chen***	***1998***	***TLCS-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
27	7523326	Octreotide	Korun	1994	i.v ethanol infusion & trauma induced	Therapeutic	No effect	Not reported
28	1719527	Octreotide	Zhu	1991	TLCS-AP	Prophylactic & Therapeutic	Mild effect	Mild effect
29	19784727	Octreotide	Guler	2009	TLCS-AP	Prophylactic	Improved	Improved
30	9115817	Octreotide	Sanchez	1997	TLCS-AP	Prophylactic & Therapeutic	No effect	No effect
31	7536997	Octreotide	Tulassay	1995	CER-AP	Prophylactic	No effect	Not reported
32	6653298	Pancreatic polypeptide	Coelle	1983	CDE diet	Prophylactic & Therapeutic	Improved	Improved
33	11741187	Peptide YY	Grise	2002	CDE diet	Prophylactic & Therapeutic	Improved	Improved
34	7685154	Peptide YY	Tito	1993	CER-AP	Prophylactic	Improved	Improved

35	2452222	Proglumide	Tarpila	1988	Intraductal infusion of bile acid & trypsin	Prophylactic	No effect	No effect
36	8592430	Sandostatin	Delany	1996	TLCS-AP	Therapeutic	Improved	Not reported
37	***9840309***	***Somatostatin***	***Berthet***	***1998***	***Pancreatic duct ligation***	***Therapeutic***	***Improved***	***Improved***
38	604191	Somatostatin	Lankish	1977	TLCS-AP	Prophylactic	No effect	No effect
39	2422635	Somatostatin & N-acetyly somatostatin agonist	Degertekin	1985	CDE diet	Prophylactic	Not reported	Not reported
40	7542690	Tetraprenylacetone	Tachibana	1995	CER-AP & TLCS-AP	Prophylactic & Therapeutic	Improved	Mild effect

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 12 - Agents affecting microcirculation tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	16183055	5-HT2A receptor antagonists - R-102444 & R-96544	Ohawa	2005	CER-AP, CDE diet and pancreatic duct ligation	Prophylactic	Improved	Improved
2	26579579	Acenocoumarol	Warzecha	2015	Ischaemia reperfusion	Prophylactic	Improved	Improved
3	***16847238***	***Activated human protein C (Drotrecogin alfa)***	***Alsfasser***	***2006***	***Caerulein + bile acid***	***Prophylactic & Therapeutic***	***Mild effect***	***No effect***
4	19680809	Activated protein C	Ping	2010	TLCS-AP	Prophylactic	Improved	Improved
5	***15987389***	***Activated protein C***	***Yamanel***	***2005***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
6	18008033	Activated protein C	Chen	2007	TLCS-AP	Prophylactic	Improved	Improved
7	***22441146***	***Activated protein C (human recombinant - Xigris)***	***Babu***	***2012***	***CER-AP***	***Prophylactic & Therapeutic***	***Improved***	***Improved***
8	17506000	Anti-factor VIIa	Andersson	2007	TLCS-AP	Prophylactic	Improved	Not reported
9	19546838	Antithrombin III	Hagiwara	2009	CER-AP	Prophylactic	Improved	Improved
10	1735347	Antithrombin III	Bleeker	1992	TLCS-AP	Prophylactic & Therapeutic	No effect	No effect
11	1384910	Bradykinin - HOE140	Griesbacher	1992	CER-AP	Prophylactic	Improved	Not reported
12	8448591	Bradykinin - HOE140	Griesbacher	1993	CER-AP	Prophylactic	Improved	Not reported
13	11786477	Bradykinin receptor antagonist - FR173657	Hirata	2002	Pancreaticobiliary duct obstruction	Prophylactic	Improved	Improved
14	8884850	Bradykinin receptor antagonist - HOE140	Kanbe	1996	Trypsin + bile acid	Prophylactic	Improved	Improved
15	2454196	Bromocriptine, amantadine & domperidone	Sikiric	1988	Pancreatic duct ligation	Prophylactic	Improved	Improved
16	25773957	Candesartan	Bostanci	2015	Caerulein + bile acid	Therapeutic	Improved	Improved
17	18948846	Danaparoid sodium	Hagiwara	2009	CER-AP	Prophylactic	Improved	Improved
18	9120115	Dextran	Schmidt	1996	Caerulein + bile acid	Therapeutic	Improved	Improved

19	6199589	Dextran 40	Crocket	1984	Closed duodenal loop	Prophylactic	No effect	Not reported
20	8783330	Diltiazem	Hughes	1996	Trypsin + Bile acid	Prophylactic	Improved	Improved
21	18086634	Dopamine	Xhang	2007	TLCS-AP	Prophylactic	Improved	No effect
22	11775728	Dopexamine	Alhan	2001	Caerulein + bile acid	Therapeutic	No effect	No effect
23	15127205	Endothelin receptor antagonist - BSF208075	Martignoni	2004	TLCS-AP and CER-AP	Prophylactic	Mild effect	No effect
24	9833805	Endothelin receptor antagonist - LU-135252	Foitzik	1998	Caerulein + bile acid	Therapeutic	Mild effect	No effect
25	9288152	Endothelin receptor antagonist - PD145065	Todd	1997	CDE diet induced	Prophylactic & Therapeutic	Improved	Not reported
26	15754391	Endothelin receptor antagonists (LU-302872,LU-302146)	Andrzejewska	2005	CER-AP	Prophylactic	Mild effect	Improved
27	14690481	Endothelin receptor antagonists (LU-302872,LU-302146)	Andrzejewska	2003	TLCS-AP	Prophylactic	Improved	Improved
28	14620534	Endothelin receptor antagonists (LU-302872,LU-302146)	Dlugosz	2003	TLCS-AP	Prophylactic	Mild effect	Improved
29	7540127	Endothelin-1	Kogire	1995	CER-AP	Prophylactic	No effect	No effect
30	15300904	Heparin	Dobosz	2004	CER-AP	Prophylactic	Improved	Not reported
31	18955758	Heparin	Ceranowicz	2008	Ischaemia - reperfusion	Prophylactic & Therapeutic	Improved	No effect
32	14739847	Heparin	Hackert	2004	CER-AP, Caerulein + bile acid, pancreatic duct obstruction and duct obstruction + ERCP	Prophylactic	variable results	different models
33	***16340748***	***Hypertonic saline***	***Machado***	***2006***	***TLCS-AP***	***Therapeutic***	***Improved***	***No effect***
34	12593711	Hypertonic saline	Shields	2001	L-arginine	Therapeutic	Improved	Improved
35	11044157	Hypertonic saline	Shields	2000	L-arginine	Therapeutic	Mild effect	Improved
36	***20351627***	***Hypertonic saline***	***Coelho***	***2010***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***No effect***

37	18695645	Kallikrein inhibitors	Griesbacher	2008	CER-AP	Prophylactic	Improved	Not reported
38	1371180	Ketanserin and Ritanserin	Oguchi	1992	CER-AP	Therapeutic	Improved	Improved
39	10632781	L-Arginine (nitric oxide donor)	Andrzejewska	1999	CER-AP	Prophylactic	Not reported	Not reported
40	7587783	L-Arginine (nitric oxide donor)	Liu	1995	CER-AP	Prophylactic	Improved	Mild effect
41	7659783	L-NAME (NG-nitro-L-arginine methyl esther) & bradykinin antagonist (HOE 140)	Closa	1995	TLCS-AP	Prophylactic	Improved	Not reported
42	***15077884***	***L-NAME & canavanine (inducible NO synthase)***	***Chen***	***2004***	***TLCS-AP***	***Therapeutic***	Improved (Canavanine)	Mild effect (Canavanine)
43	18167214	Low molecular weight heparin	Qiu	2007	TLCS-AP	Therapeutic	Improved	Not reported
44	10732291	Mixed endothelin receptor antagonist - Bosentan	Fiedler	1999	TLCS-AP	Therapeutic	No effect	No effect
45	19451746	Monoclonal antibodies of P-selectin	Hackert	2009	Caerulein + bile acid	Prophylactic	Mild effect	Improved
46	19826187	Obestatin	Ceranowicz	2009	CER-AP	Prophylactic	Improved	Improved
47	6422906	Oxidopamine	Donahue	1984	TLCS-AP	Prophylactic	Not reported	Not reported
48	15782104	Oxyglobin	Strate	2005	Caerulein + bile acid	Prophylactic	Improved	Improved
49	14578741	Oxyglobin	Strate	2003	Caerulein + bile acid	Prophylactic	Improved	Improved
50	16570348	Relaxin	Binker	2006	Closed-duodenal-loop	Prophylactic & Therapeutic	Improved	Improved
51	18832108	Risperidone	Yamaguchi	2009	CDE diet	Prophylactic	Mild effect	Improved
52	15582722	Thyrotropin releasing hormone (RX 77368)	Yoneda	2005	CER-AP	Prophylactic	Improved	Improved
53	7587813	Verapamil	Lake	1995	CDE diet induced	Prophylactic	Not reported	Improved

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 13 - Eicosanoids tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	17511027	Benzenesulfonamide	Seo	2007	CER-AP	Prophylactic	Improved	Improved
2	***16134005***	***Celecoxib***	***Alhan***	***2004***	***Caerulein + bile acid***	***Therapeutic***	***Improved***	***Improved***
3	12381531	Celecoxib & NS-398	Song	2002	CER-AP	Prophylactic	Improved	Mild effect
4	16995470	Cyclooxygenase & 5 lipoxygenase inhibitor - ER-34122	Kalyoncu	2006	CER-AP	Prophylactic	Improved	Improved
5	20977452	Flavocoxid	Polito	2010	CER-AP	Prophylactic	Improved	Improved
6	***15088642***	***Iloprost***	***Dlugosz***	***2004***	***Intragastric ethanol and TLCS-AP***	***Therapeutic***	***Mild effect***	***Mild effect***
7	2599282	Indomethacin	Wilderhain	1989	CDE diet	Prophylactic	Improved	Improved
8	11321505	Indomethacin, refecoxib, resveratrol & LPS	Jaworek	2001	CER-AP	Prophylactic	Improved	Improved
9	7690378	Lipoxygenase inhibitor - AA-861	Kiriyama	1993	TLCS-AP	Prophylactic	Mild effect	Mild effect
10	19295455	Omega 3 fatty acids	Kilian	2009	Caerulein + bile acid	Therapeutic	Improved	No effect
11	16785730	Omega 3 fatty acids	Alhan	2006	Caerulein + bile acid	Therapeutic	Improved	Improved
12	***16924320***	***Parecoxib***	***Almeida***	***2006***	***TLCS-AP***	***Therapeutic***	***Improved***	***No effect***
13	9635806	Phospholipase A2 inhibitor - BM 16.2056	Uhl	1998	CER AP & TLCS-AP	Prophylactic & Therapeutic	Improved	Improved
14	15467263	Phospholipase A2 inhibitor - S-5920/LY315920Na	Tomita	2004	TLCS-AP and intraductal PLA2	Prophylactic & Therapeutic	Improved	Improved
15	10438167	Phospholipase A2 inhibitor - S-5920/LY315920Na	Yoshikawa	1999	Trypsin + bile acid	Therapeutic	Improved	Mild effect
16	***20963474***	***Polyunsaturated FA: N3, N6, N9 fatty acids***	***Kilian***	***2011***	***Bile acid induced***	***Therapeutic***	***No effect***	***No effect***

17	9211498	Prostacyclin analogue - P-thiaiminoprostacyclin	Dlugosz	1997	Bile acid and ethanol (intra-gastric)	Prophylactic	Improved	Improved
18	1486188	Prostagandins (MR-356, PGE1 oligomer)	Sakai	1992	Bile acid + trypsin	Prophylactic & Therapeutic	Improved	Improved
19	3550789	Prostaglandins (PG E2 & I2)	Olazábal	1986	Bile acid induced	Prophylactic & Therapeutic	No effect	No effect
20	1282294	Prostaglandins (PGE1 & PGE 2) & protease inhibitor - ONO3307	Hirano	1992	CER-AP	Prophylactic	Mixed effect	Mixed effect
21	1690420	Prostaglandins (PGE1)	Buscail	1990	CER-AP	Prophylactic	Improved	Improved
22	11995492	Prostaglandins (PGE1)	Yücel	2002	Caerulein + bile acid	Therapeutic	Mild effect	Improved
23	8720663	Prostaglandins (PGE1)	Pozsar	1996	Closed duodenal loop	Prophylactic	Improved	Improved
24	15028970	Zafirlukast	Oruc	2004	CER-AP	Prophylactic	Improved	Mild effect

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

Table 14 - Other agents tested in EAP

	PMID	Agent	1st author	Year of publication	Method of inducing pancreatitis	Prophylactic or Therapeutic	Effect on biochemical parameters	Effect on histological parameters
1	12409834	Adenosine uptake inhibitors	Noji	2002	CER-AP	Prophylactic	Improved	Improved
2	25604657	Astragaloside IV	Qiu	2015	TLCS-AP and L-arginine	Prophylactic	Improved	Improved
3	***26393905***	***Baicalein***	***Li***	***2015***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***Improved***
4	***17278194***	***Baicalin***	***Zhang***	***2007***	***TLCS-AP***	***Therapeutic***	***Mild effect***	***Improved***
5	19657312	Baicalin	Zhang	2009	TLCS-AP	Therapeutic	Improved	Improved
6	20038432	Betacellulin	Dahlhoff	2010	CER-AP and L-Arginine	Prophylactic	Improved	Improved
7	***18571584***	***Bismethylspermine***	***Jin***	***2008***	***TLCS-AP***	***Prophylactic & Therapeutic***	***Mild effect***	***No effect***
8	***26642860***	***Caffeine***	***Huang***	***2015***	***TLCS, CER- AP and FAEE-AP***	***Prophylactic& Therapeutic***	***Improved***	***Improved***
9	24817315	Chaiqin Chengqi Decoction	Guo	2015	L-arginine	Prophylactic	Improved	Improved
10	23892997	Clotrimazole	Cekic	2013	Caerulein + bile acid	Therapeutic	Improved	Improved
11	23956866	Diazepam	Abed	2013	CER-AP	Prophylactic	Improved	Improved
12	25486529	Dimethyl fumarate	Robles	2015	L-arginine	Prophylactic	Improved	Improved
13	25351888	Emodin	Yao	2015	TLCS-AP	Therapeutic	Improved	Improved
14	***15810074***	***Emodin & baicalin***	***Zhang***	***2005***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
15	10394025	Epidermal growth factor	Warzecha	1999	CER-AP	Prophylactic	Improved	Improved
16	19333535	Erythropoietin	Ucan	2009	TLCS-AP	Therapeutic	Mild effect	Improved
17	26646279	Fetal membrane derived mesenchymal stem cells	Kawakubo	2015	Bile acid	Therapeutic	Improved	Improved
18	16718788	Fibroblast growth factor	Yan	2006	CER-AP	Prophylactic	Improved	Improved
19	10824691	Fibroblast growth factor	Hosokawa	2000	CER-AP and TLCS-AP	Therapeutic	Mild effect	Mild effect
20	20662791	Galanin receptor antagonist:	Bareto	2010	CER-AP	Prophylactic	Improved	Mild effect

		Galanitide						
21	21242707	Galanin receptor antagonists: C7, M35,M40 & galanitide	Bhandari	2010	CER-AP	Prophylactic	Improved (M35 & Galantide)	Improved (M35 & Galantide)
22	19506532	Ghrelin	Zhou	2009	TLCS-AP	Prophylactic	Improved	Improved
23	***20814069***	***Ghrelin***	***Warzecha***	***2010***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***
24	25594510	Ghrelin	Bukowczan	2015	Ischemia reperfusion	Therapeutic	Improved	Improved
25	25145902	Glutamine	Alhan	2015	Caerulein + Bile acid	Therapeutic	Improved	Improved
26	***24302175***	***Glycyrrhizin***	***Yildrim***	***2013***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
27	***25917787***	***GSK7975-A and CM_128***	***Wen***	***2015***	***TLCS-AP, CER-AP and FAEE-AP***	***Therapeutic***	***Improved***	***Improved***
28	11698071	Hepatocyte growth factor	Warzecha	2001	CER-AP	Prophylactic	Improved	Improved
29	25142942	Human bone marrow-derived clonal mesenchymal stem cells	Jung	2015	CER + LPS	Therapeutic	Improved	Improved
30	24294357	Human umbilical cord-derived mesenchymal stem cells	Meng	2013	TLCS-AP	Therapeutic	Improved	Improved
31	23678547	Lawsone	Birudar	2013	L- Arginine	Therapeutic	Improved	Improved
32	26100532	L-Lysine	Al-Malki	2015	L-Arginine	Prophylactic & therapeutic	Prophylactic better than therapeutic	Prophylactic better than therapeutic
33	22079843	Lycopene	Ozkan	2012	CER-AP	Prophylactic	Improved	Improved
34	22687382	Melatonin	Huai	2012	TLCS-AP	Prophylactic	Improved	Improved
35	20210857	Melatonin	Jung	2010	CER-AP	Prophylactic	Improved	Improved
36	19454824	Melatonin	Col	2009	Pancreatic duct ligation	Therapeutic	Improved	Not reported
37	16499554	Melatonin	Munoz-Casares	2006	Ischaemic reperfusion	Prophylactic & Therapeutic	Improved	Improved
38	10573371	Melatonin	Qi	1999	CER-AP	Prophylactic	No effect	Improved
39	22575522	ND-07 (antioxidant & anti-inflammatory)	Lee	2012	Caerulein + LPS	Prophylactic	Improved	Improved
40	25912801	Obestatin	Bukowczan	2015	Ischemia reperfusion	Prophylactic	Improved	Improved
41	***26474436***	***Obestatin***	***Bukowczan***	***2015***	***CER-AP***	***Therapeutic***	***Improved***	***Improved***

42	26226277	Obestatin	Bukowczan	2015	Ischemia reperfusion	Therapeutic	Improved	Improved
43	25579844	Orlistat	Patel	2015	CER-AP	Therapeutic	Improved	Improved
44	18688721	Pentoxifylline	Gül	2009	CER-AP	Prophylactic	Improved	Improved
45	9561559	Pentoxifylline	Marton	1998	TLCS-AP	Prophylactic	Mild effect	No effect
46	7988133	Pentoxifylline	Bassi	1994	Caerulein + Bile acid	**Therapeutic**	No effect	No effect
47	***19684433***	***Pentoxifylline***	***Matheus***	***2009***	***TLCS-AP***	***Therapeutic***	***Improved***	***Improved***
48	***22760835***	***Pentoxifylline***	***Coelho***	***2012***	***TLCS-AP***	***Therapeutic***	***Improved***	***No effect***
49	20599924	Pentoxifylline & alpha lipoic acid	Abdin	2010	L-arginine	Prophylactic	Improved	Improved
50	23275617	Pepducin lipopeptide - P2pal-18S	MIchael ES	2013	CER-AP	Prophylactic & Therapeutic	Mild effect	Mild effect
51	***25455348***	***Percutaneous catheter drainage***	***Chen***	***2015***	***Bile acid***	***Therapeutic***	***Improved***	***Improved***
52	1700770	Peritoneal dialysis	Tilquin	1990	Intraductal trypsin infusion	Therapeutic	Not reported	Not reported
53	25893772	Peritoneal drainage	Zhu	2016	Bile acid	Therapeutic	Improved	Improved
54	***25760428***	***Polyenoylphosphatidylcholine***	***Li***	***2015***	***Bile acid***	***Therapeutic***	***Improved***	***Improved***
55	25834318	Qingyi	Zhang	2015	Bile acid	Prophylactic	Improved	Improved
56	20823570	Quercetin	Carralho	2010	CER-AP	Prophylactic	Improved	Improved
57	26683606	Recombinant Reg3α protein	Yu	2016	L-Arginine	Therapeutic	Improved	Improved
58	25266882	S100A12 (calcium binding protein)	Feng	2015	CER-AP and Caerulein + LPS	Prophylactic	Improved	Improved
59	25001186	Trimetazidine	Tanoglu	2015	CER-AP * 4	Therapeutic	Improved	Improved
60	***26071131***	***TRO40303 and DEB025***	***Mukherjee***	***2015***	***TLCS, CER-AP and FAEE-AP***	***Therapeutic***	***Improved***	***Improved***
61	26608189	Ursodeoxycholic acid	Katona	2016	Bile acid	Prophylactic	Improved	Improved

Highlighted studies are the ones which fulfilled criteria for most clinically relevant and objective parameters of assessment from scoring system

3.4 Discussion

Successful pre-clinical assessment of any therapeutic regimen depends on the reliability and reproducibility of experimental models used (Hay, Thomas et al. 2014). Experimental models of AP have been developed to study the physiology and pathogenesis of disease and test new therapies. Rodents have been the most commonly used species as murine models of AP are standardized, relatively inexpensive, can be manipulated genetically to create knockout or transgenic animals and reagents such as anti-bodies are readily available (Lerch and Gorelick 2013). However it is important to note that the natural history of disease in rodents may not be completely representative of human pathophysiology and it is therefore important to choose the most appropriate model for answering the specific scientific question. This is even more relevant in the context of testing therapies in pre-clinical models of AP as the lack of clinically relevant and predictive models has been identified as a significant limitation in the development of an effective therapeutic for other acute conditions such as sepsis (Fink 2014) and stroke (Sena, van der Worp et al. 2007). In this analysis, caerulein was the most commonly used toxin for induction of AP and testing new treatments in pre-clinical studies. Although the secretagogue model of AP is an adequate model for examining the pathobiology of disease and evaluate intracellular signaling, protease activation pathways as well as cell death pathways; it induces a mild form of pancreatitis. An agent's therapeutic efficacy should therefore be tested in a severe form of clinically representative model of EAP, such as bile acid or alcoholic pancreatitis before it is considered as a treatment for human AP. It is also

important to highlight the significant variation in the severity of CER-AP and bile acid induced AP models in the published literature, both in terms of the dose and duration of exposure to toxin, as demonstrated in figure 3.3 (A-D). The other significant findings are that in approximately 60% of published studies, agents have been evaluated as a prophylactic treatment and in 88.4%; therapeutic efficacy has been investigated in a single model of EAP. A thorough evaluation of any potential agent that is being considered for treatment of clinical AP should therefore focus on treating a specific biological response, demonstrate a clearly defined mechanism of action and show that a proposed treatment is effective in attenuating the pathobiological response across several animal models when administered therapeutically. Some researchers have even advocated the use of 'co-clinical trials', in which mice suffering from a disease are treated in a 'mouse clinical space' in the same way as human patients are treated in an ordinary hospital. It is, however, important to note that pre-clinical studies have provided useful clinical parallels for treatment post-ERCP induced clinical AP and translation of Indomethacin (Foitzik, Hotz et al. 2003, Elmunzer, Scheiman et al. 2012) from bench to bedside which highlights the importance of carefully designed pre-clinical studies focusing on clinically relevant models.

A majority of agents that have been tested pre-clinically have been shown to be effective in reducing disease severity (tables 8-14) and very few negative results have been published. This may point towards publication bias, as it is known that laboratory based experimental studies are more susceptible to publication bias compared with randomised clinical trials (Easterbrook, Berlin

et al. 1991). If positive, rather than neutral studies are published, then any conclusions drawn from the published literature would overstate the magnitude of any effect seen, which may be the case in therapies tested in EAP. There was also a significant lack of consistency in reporting both the biochemical and histological outcomes because of which a combined quantitative analysis / meta-analysis could not be undertaken. The use of ARRIVE guidelines (Kilkenny, Browne et al. 2010) could provide a standardized approach for reporting *in vivo* studies of AP.

Attempts at inhibition of pancreatic secretion, auto digestion, inflammatory pathways, reduction of oxidant free radicals and improving microcirculation have been unsuccessful in clinical trials for the therapeutic treatment of AP thus far. It is, however, interesting to note that few agents have primarily targeted PAC injury – which is probably the cardinal event in the pathophysiology of AP. This highlights the need to identify new molecular targets and develop novel compounds for the treatment of AP. Alternatively, there has been increasing interest in repositioning of existing drugs for new indications from pharmaceutical as well as biotech companies over the last two decades (Ashburn and Thor 2004) and repositioning could be explored as an innovative strategy of drug discovery for AP in the future.

Unlike clinical trials where instruments such as the Jadad score (Jadad, Moore et al. 1996) and CONSORT guidelines (Schulz, Altman et al. 2010) can be used to assess the quality of randomised clinical trials, there are no specific tools available to evaluate the quality of experimental studies. Researchers in stroke have developed a checklist, Collaborative Approach to Meta-Analysis

and Review of Animal Data in Experimental Stroke (CAMARADES) (Macleod, O'Collins et al. 2005) to address this issue and in an endeavour to evaluate the quality of published studies a new, pancreas specific scoring system has been developed and used as a standard to evaluate pre-clinical studies. The majority of agents included in this review demonstrated efficacy in ameliorating the severity of EAP, yet there is no targeted therapy for human AP due to the subsequent failure of all investigational medicinal products in clinical trials. Analysis of the published literature testing therapeutic efficacy in EAP using the newly devised scoring system highlights the heterogeneity in the quality of studies and lack of clinically relevant experimental designs. Only 15.3% (66 out of 430) fulfilled the most objective and clinically relevant modified criteria and of these only fourteen agents reduced severity of biochemical and histological markers of severity by > 50% (table 5). These findings enable us to identify potential deficiencies on the bench that would contribute to translational inefficiency of developing a treatment for AP on bedside.

Although designing and conducting high quality clinical trials for AP has proved challenging due to the delay in presentation of patients, lack of highly sensitive and specific measures of predicting severity of disease and having outcomes that would demonstrate clinical as well as statistical significance (Mason and Siriwardena 2005); there is room for innovative and adaptive trial design to bridge this translational gap. Reducing the door to needle time and providing treatment at an earlier time point could have a significant impact on the outcome of clinical trials. There is a pressing need for well-developed, multi-disciplinary clinical research infra-structure and national as well as

international networks to conduct high quality clinical trials. The international symposium regarding the progress and challenges of AP (Afghani, Pandol et al. 2015) is one such effort; however collaboration between industry, academia, regulatory agencies and governments alongside patient involvement would be crucial to meet the challenges for therapy development of AP.

The present review provides a consolidated resource of a wide variety of compounds that have been evaluated for the treatment of EAP, summarizing their modes of action and also provides a novel and in depth qualitative analysis of published literature. Preclinical studies demonstrated efficacy of inhibitors of secretion and digestive enzymes, anti-oxidants, metabolic, circulatory and immunological agents, but few have targeted primary acinar cell injury and only modest qualitative improvements in studies over time. While attrition is expected in drug development, our findings identify a significant translational gap between animal studies and clinical trials in AP. We propose standardised reporting of preclinical studies to improve the quality, comparability and translation of EAP treatment. To improve the translational efficiency, investigators will need to identify more suitable therapeutic targets, improve pre-clinical trial design and their approaches for selecting candidate compounds for clinical development and adopt better designs for clinical trials.

4. Chapter 4 – Proof of concept: effects of Cyclophilin D inhibition on mitochondrial dysfunction and necrotic cell death pathway activation in murine and human pancreatic acinar cells

4.1 Introduction

The physiology of PAC mitochondrial function and the role of mitochondrial dysfunction secondary to MPTP formation leading to diminished ATP levels has been discussed in detail in section 1.4.

Bile acids are steroid acids found predominantly in the bile of mammals. Bile salts are bile acids compounded with a cation, usually sodium. These bile acids are synthesised by the hepatocytes by cytochrome P450-mediated oxidation of cholesterol. They are conjugated either with the amino acids taurine or glycine, or with a sulphate or glucuronide and stored in the gall bladder where they are concentrated by removal of water (Bennett 1964). The two primary bile acids in humans are cholic acid and chenodeoxycholic acid (Kakiyama, Pandak et al. 2013), the latter being the common form. Chenodeoxycholic acid is dehydroxylated by intestinal bacteria by removal of 7-alpha hydroxyl group resulting in the formation of lithocholic acid (litho=stone); which only has a 3-alpha hydroxyl group, and is poorly water soluble and toxic to cells (Ridlon, Kang et al. 2006). Bile acids synthesised in the liver are termed as ‘primary’ bile acids, and those made by bacteria are referred to as ‘secondary’ bile acids. Chenodeoxycholic acid is therefore a primary bile acid and lithocholic acid a secondary bile acid. Taurolithocholic acid 3-sulphate (TLCS) is a well characterised bile acid that can induce PAC Ca^{2+} oscillations and at higher doses lead to pathological Ca^{2+}, loss of mitochondrial membrane potential ($\Delta\psi_m$) and reduced ATP levels (Voronina, Longbottom et al. 2002, Voronina, Barrow et al. 2004, Voronina, Barrow et al. 2010). The other common taurine-conjugated bile acids include Taurocholic acid and

Taurochenodeoxycholic acid which can also trigger similar local as well as global calcium signals, however these require higher doses in millimolar concentrations, compared to micromolar concentrations required with TLCS (Voronina, Longbottom et al. 2002).

Non oxidative metabolism of ethanol and fatty acids by synthases, abundantly found in PACs (Lange and Sobel 1983), leads to the formation of fatty acid ethyl esters (FAEEs). These FAEEs cause cytosolic calcium overload in PAC via activation of IP3 receptors and inhibition of ATP production which results in SERCA and PMCA pump failure (Criddle, Murphy et al. 2006) rather than release from ER Ca^{2+} channels. Both palmitoleic acid ethyl ester (POAEE) and its hydrolytic product palmitoleic acid (POA) have the ability to uncouple oxidative phosphorylation, an effect independent of mitochondrial depolarisation, and exhibit their toxic effects on PACs (Booth, Murphy et al. 2011, Samad, James et al. 2014). Protection of cells from this sequence can be provided by excluding Ca^{2+} from the external medium (Raraty, Ward et al. 2000, Criddle, Raraty et al. 2004) or providing supplementary intracellular ATP (Criddle, Murphy et al. 2006). This has been demonstrated in patch clamp experiments where 4 mM ATP supplied intracellular via patch pipette, has shown to maintain the activity of SERCA and PMCA pumps to avoid pathological, global, sustained elevations of Ca^{2+} (see figure 4.1). Insulin pre-treatment has also shown to prevent the POA induced Ca^{2+} overload, ATP depletion and cell death via necrosis in rat PACs (Samad, James et al. 2014). The pancreas produces more FAEE than any other organ in the human body

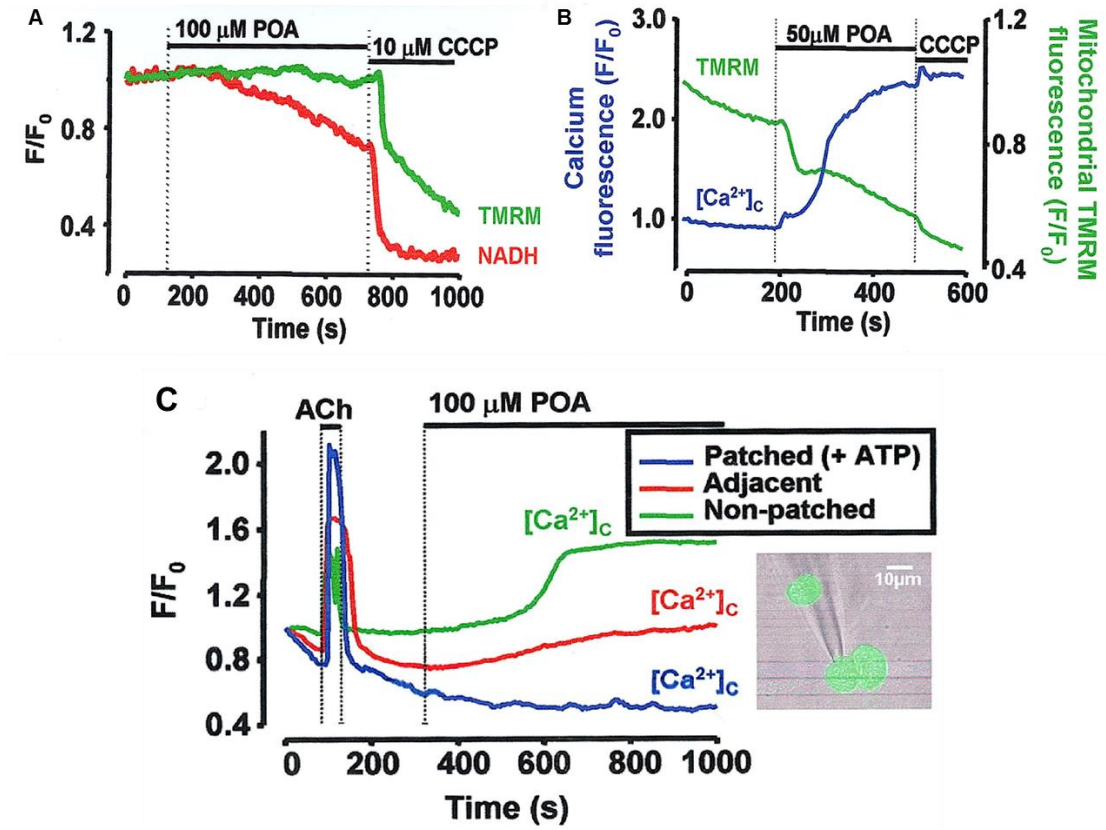


Figure 4.1 - PAC mitochondrial impairment induced by POA

(A) Fall in NADH autoflourescence after exposure to POA in a cell pre-treated with a Ca^{2+} chelator results in minimal change in Ca^{2+} , or fall in $\Delta\psi_m$ suggesting diminished ATP production without an immediate loss in electrochemical gradient (B) Large sustained rise in Ca^{2+} in response to POA application is seen in cell not treated with Ca^{2+} chelator and accompanied by a fall in $\Delta\psi_m$ (C) POA induces a pathological, sustained rise in Ca^{2+}, that is prevented by adding ATP to the cytosol by patch pipette for calcium clearance. An adjacent cell, that can receive some ATP via gap junctions, shows a dampened rise, not as significant as the non-patched cell. *Modified from (Criddle, Murphy et al. 2006)*

(Laposata and Lange 1986, Gukovskaya, Mouria et al. 2002) and therefore FAEE mediated toxicity plays an important role in the pathophysiology of AP. Importantly though, agents that cause pancreatitis - TLCS, POAEE, POA - all lead to reduced cytosolic and mitochondrial ATP (Voronina, Barrow et al. 2010).

Pharmacological and genetic deletion of CypD inhibits MPTP formation and is shown to preserve mitochondrial function and reduce cell death in murine PACs in response to CCK and TLCS (Mukherjee, Mareninova et al. 2015). The protective effects of CypD inhibition in response to POA however have not been characterised. This chapter describes experiments that examine the relevance of murine findings with protective effects of CsA and DEB025 on TLCS induced necrotic cell death in human PACs and the protective effects of CypD deletion on POA induced mitochondrial dysfunction. As a prelude to undertaking these experiments, the findings of CypD inhibition using CsA and cells isolated from Ppif-/- mice were replicated as proof of concept.

4.2 Methods

All experiments were undertaken on freshly isolated PACs as described in section 2.3. Samples of normal pancreas from patients undergoing surgery for left-sided or non-obstructing pancreatic head tumours were used for these experiments. The time from sampling to the start of cell isolation was less than 10 minutes in every case to ensure high quality cells. Mitochondrial membrane potential ($\Delta\psi_m$), NAD(P)H autoflourescence and necrotic cell death assays were performed as previously described in sections 2.43 and 2.44 respectively. Details of the standard PCR assay used for genotyping of Ppif-/- mice are described in section 2.5.

4.3 Results

4.3.1 Confirmation of CypD knockout in Ppif -/- colony

Genotyping confirmed the absence of CypD in PAC homogenates extracted from Ppif-/- mice and was detected in wild type control mice. Blots were repeated in triplicate with a representative blot demonstrated (see figure 4.2).

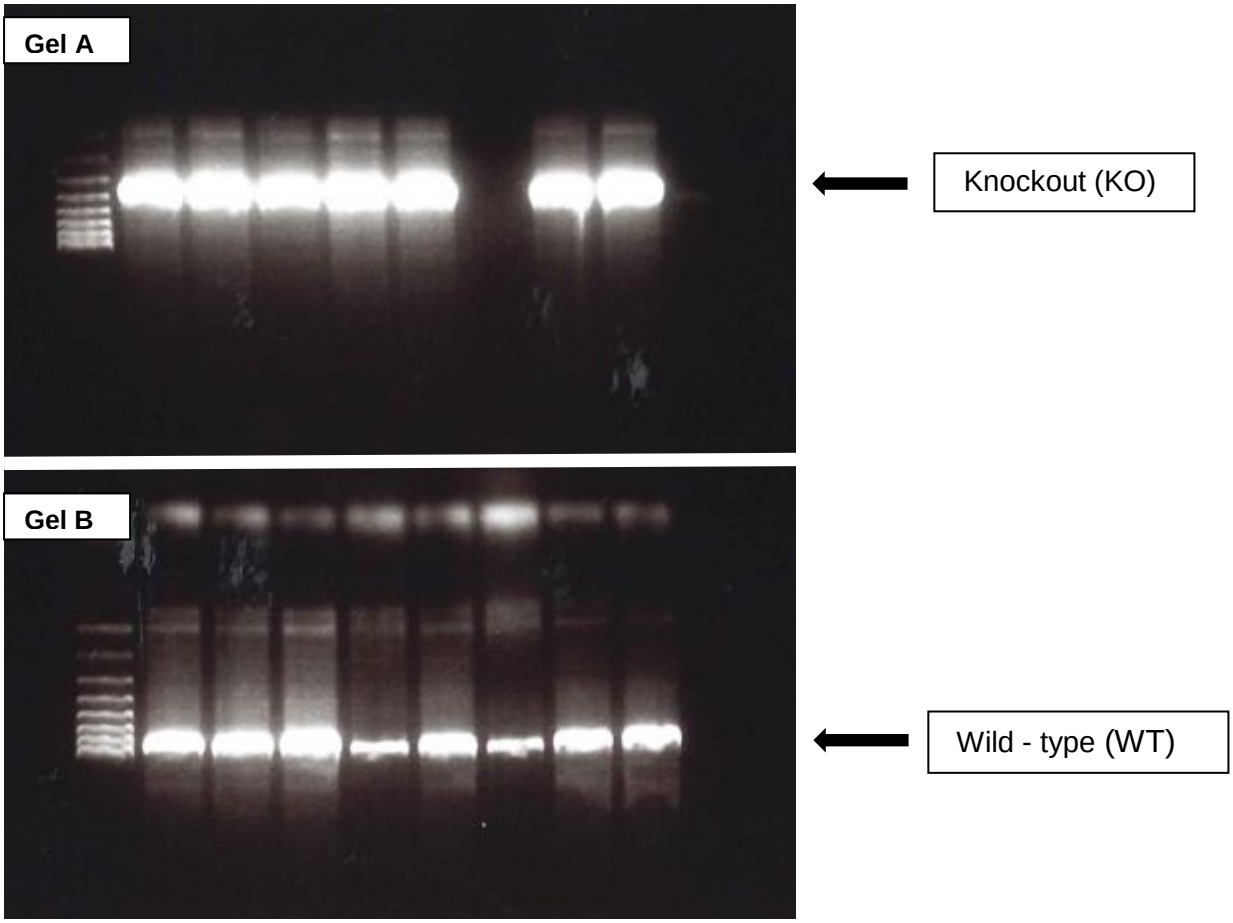


Figure 4.2 - PCR confirming the absence of CypD in Ppif -/- mice

Cyclophilin D (~ 300 bp) is not detected in PAC homogenates extracted from Ppif-/- strain of mice and is detected in strain matched controls. The band ~900 bp in the bottom gel B represents the wild type mouse and the band ~600 bp in the top gel A represents the knockout mouse.

Note: Empty band in lane 6 (left to right) of Gel A is a loading artefact

4.3.2 Pathological acinar mitochondrial membrane potential responses to TLCS with CypD knockout

Changes in $\Delta\psi_m$ were recorded in freshly isolated murine PACs using confocal fluorescence microscopy. Cells from wild type controls showed significant loss of $\Delta\psi_m$ in response to pathological concentration of TLCS (500 µM) (red trace fiigure 4.3A and top panel 4.3B). Cells isolated from Ppif -/- animals maintained $\Delta\psi_m$ when perfused with TLCS (500 µM) (blue trace figure 4.3 and bottom panel 4.3B). The Ppif -/- cells did not show any difference in maximal depolarisation compared to controls, when CCCP (10 µM) – a protonophore that uncouples the proton gradient and oxidative phosphorylation – was applied at the end as an internal control.

.

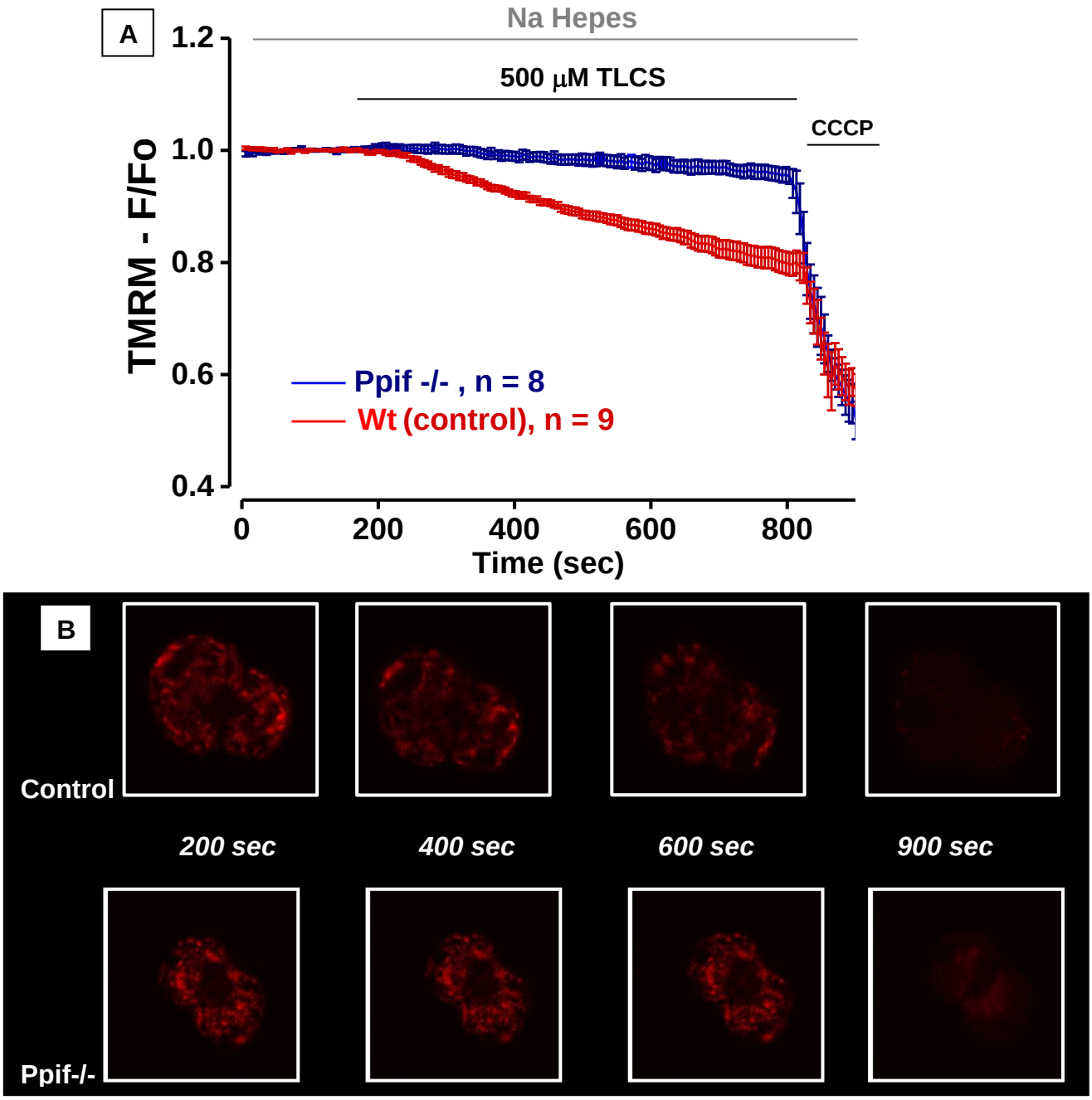


Figure 4.3 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in Ppif -/- and Wt mice

Mean changes in TMRM fluorescence, from at least three biological replicates (n=number of cells), over baseline are shown (F/Fo) +/- SEM, with respective number of recordings shown in the key (A). $\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation (top panel 4.3B) in acinar cells isolated from WT mice with complete depolarisation on application of protonophore CCCP, used as a positive control. PACs isolated from Ppif-/- displayed a markedly reduced depolarisation in response to TLCS, preserving $\Delta\psi_m$ (bottom panel 4.3B).

4.3.3 Pathological acinar mitochondrial membrane potential responses and NAD(P)H to POA + EtoH with CypD knockout

Changes in NAD(P)H were measured by detecting autofluorescence of the isolated PACs using confocal fluorescence microscopy. NAD(P)H is produced during the tricarboxylic acid cycle and provides energy for cellular functions. NADH levels are an indicator of mitochondrial metabolism and a surrogate marker of ATP levels within the cell. A combination of ethanol (10 mM) and POA (20 μM) induced mitochondrial depolarisation (red trace figure 4.4A) and a profound reduction of NAD(P)H autofluorescence (red trace figure 4.4B) in the mitochondrial perigranular region, indicative of mitochondrial inhibition in PACs isolated from wild type animals. NAD(P)H (blue trace figure 4.4B) changes and $\Delta\psi_m$ (blue trace figure 4.4A) are significantly reduced in PACs from Ppif -/- mice suggesting prevention of mitochondrial dysfunction in the absence of CypD.

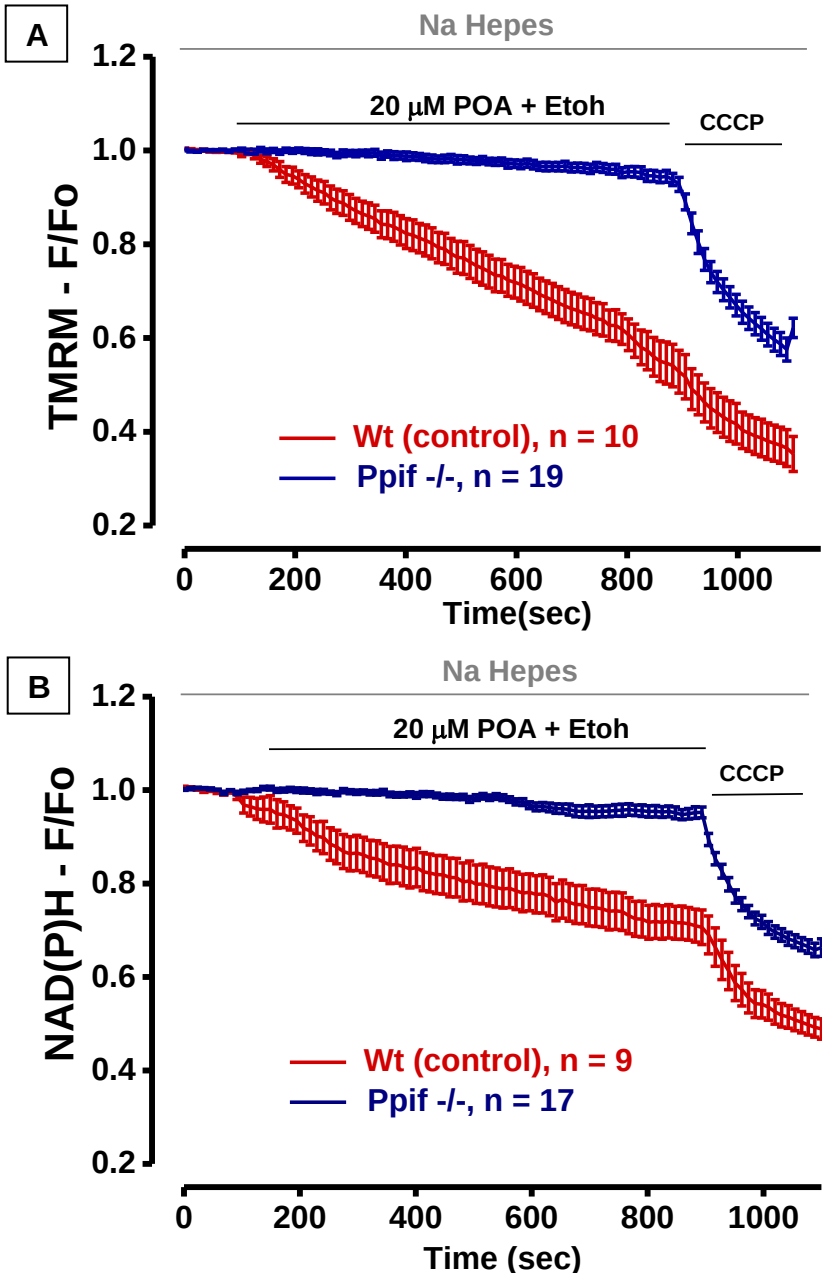


Figure 4.4 - Pathologic PAC $\Delta\psi_m$ and NAD(P)H responses to POA and ethanol, in Ppif -/- and Wt mice

$\Delta\psi_m$ changes in response to POA (20 μM) and ethanol (10 mM) show significant depolarisation and reduction in NAD(P)H levels in acinar cells isolated from WT mice with complete depolarisation on application of protonophore CCCP, used as a positive control. PACs isolated from Ppif-/- displayed reduced depolarisation and fall in NAD(P)H levels in response to POA and ethanol, preserving mitochondrial function. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) +/- SEM, with respective number of recordings shown in the key.

4.3.4 Acinar mitochondrial membrane potential responses to TLCS in the presence of CsA

Chemical inhibition of MPTP using CsA (10 μM), non-selective cyclophilin inhibitor, showed a similar profile of markedly reduced loss of $\Delta\psi_m$ in response to pathological concentration of TLCS (500 μM) (blue trace figure 4.5) similar to cells isolated from Ppif -/- animals (blue trace figure 4.3 and bottom panel 4.3). Cells pre-treated with CsA did not show any difference in maximal depolarisation compared to controls, when CCCP (10 μM) was applied at the end as an internal control.

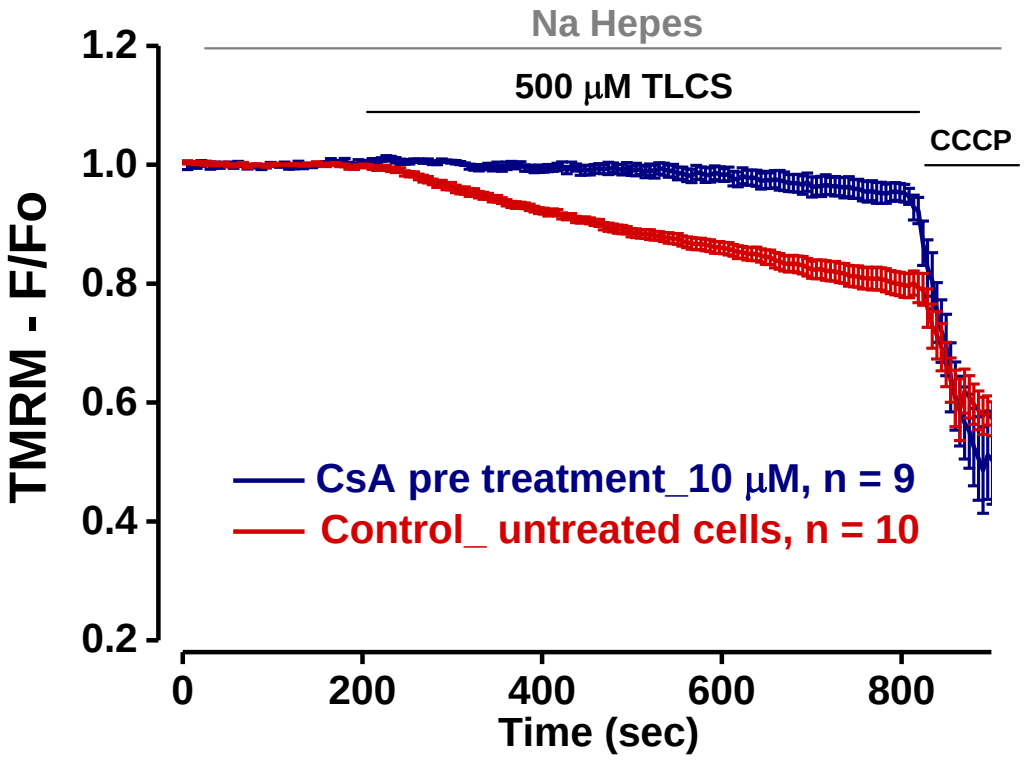


Figure 4.5 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of CsA

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with CsA (10 μM) decreased the level of depolarisation, preserving $\Delta\psi_m$. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) +/- SEM, with respective number of recordings shown in the key.

4.3.5 Necrotic cell death responses to TLCS in murine and human pancreatic acinar cells in the presence of CsA and DEB025

CsA inhibits CypD, but also binds to CypA, to form a complex that binds to calcineurin, suppressing transcription and immune responses. D-MeAla^3-EtVal^4- cyclosporine (Alisporivir, DEB025) is a non-immunosuppressive cyclosporine derivative with a strong affinity for CypD that does not have significant affinity for calcineurin, hence avoiding immunosuppressive effects. In order to evaluate the protective effects of MPTP inhibition by targeting CypD on activation of necrotic cell death pathway, necrosis assays were conducted on murine as well as human PACs. Freshly isolated acinar cells were exposed to TLCS (500 µM) in the presence or absence of CypD inhibitors CsA (10 µM in murine PAC and 100 nM in human PAC) and DEB025 (100nM). Activation of necrotic cell death pathway was detected by PI uptake - a marker of plasma membrane disruption. Cells were untreated in the control group, treated with TLCS (500 µM) in the TLCS group and co-incubated with MPTP inhibitors (CsA or DEB025) and TLCS for 30 minutes. MPTP inhibition with CsA (10 µM) significantly reduced necrosis in murine PAC (figure 4.6A). In human PACs, both chemical inhibitors of MPTP (CsA and DEB025 at 100 nM) significantly reduced activation of necrotic cell death pathway (figure 4.6B).

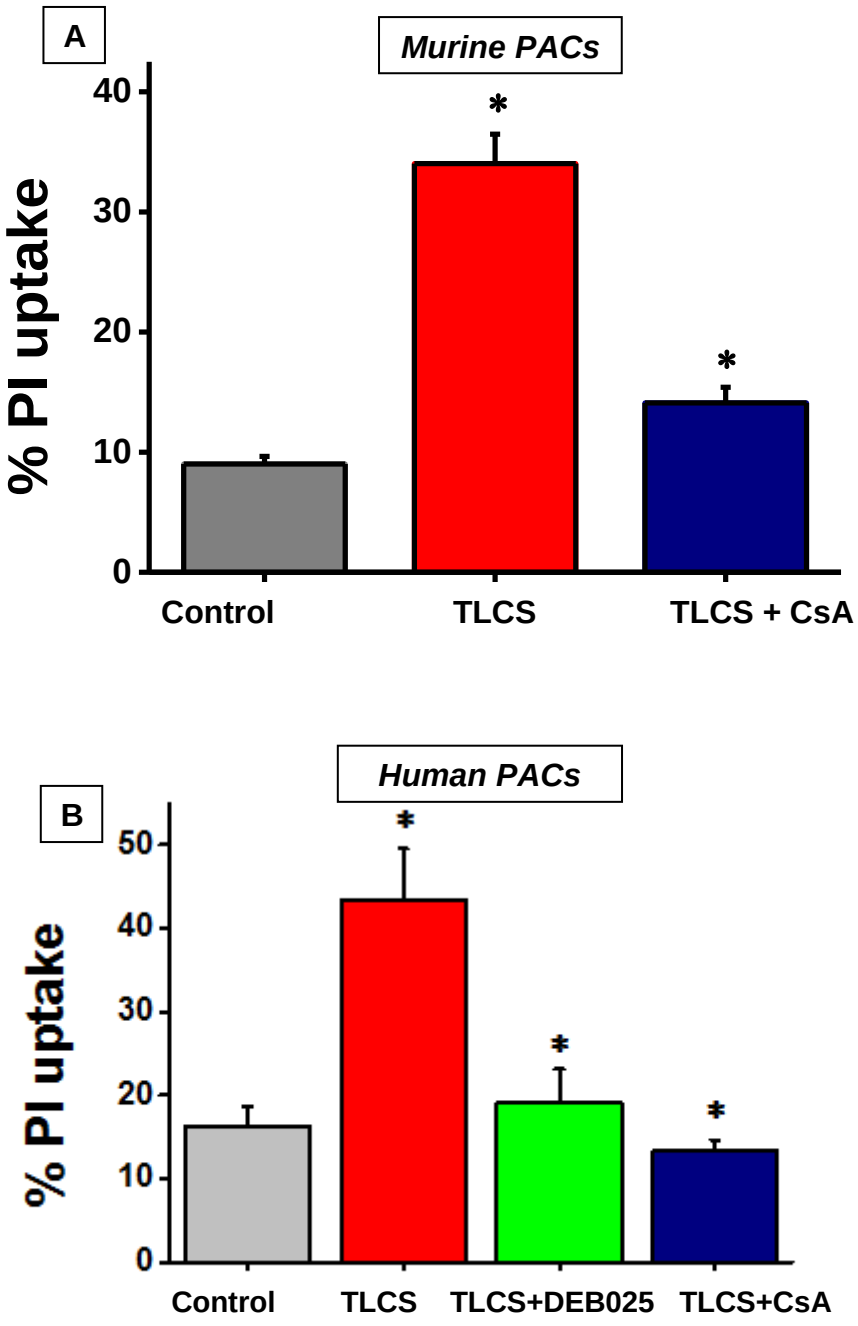


Figure 4.6 - Necrotic cell death responses to TLCS in the presence or absence of CypD inhibitors

Percentage cells showing PI uptake indicative of early plasma membrane rupture. * $p<0.05$, effect of uninhibited toxins vs no toxin vs TLCS with inhibitor. CsA protected murine cells from plasma membrane disruption. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (A). Both CsA and DEB025 protected human PACs from plasma membrane disruption. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three human pancreatic samples (B).

4.4 Discussion

Lack of experimental data on human pancreatic specimens has been a major limitation of previous experimental studies and this important aspect has been addressed in the present study. These data support the notion that isolated PAC responses are similar in murine and human cells (Murphy, Criddle et al. 2008). Significant reduction in necrotic cell death in response to TLCS observed in freshly isolated human PACs, pre-treated with CsA and DEB025 support the translatability of these findings. These data suggest that other murine findings concerning the MPTP could translate to human AP as well, but clearly further experiments examining the effects of MPTP inhibition in high quality human pancreatic samples would be required.

The exocrine pancreas uses both oxidative as well as non-oxidative metabolism of ethanol to degrade alcohol. The non-oxidative metabolism pathway yields the lipophilic FAEEs due to the combination of ethanol with fatty acids (Gukovskaya, Mouria et al. 2002) which are potent pancreatic toxins as previously described. The preservation of mitochondrial function in response to POA + ethanol application is a novel and important result. These findings demonstrate the importance of CypD inhibition in mitochondrial protection from pancreatic toxicity induced by non-oxidative metabolism of ethanol. Subsequent *in vivo* experiments (Mukherjee, Mareninova et al. 2015) revealed that genetic MPTP inhibition in Ppif-/- mice markedly reduced the biochemical and histological responses of alcoholic pancreatitis (Huang, Booth et al. 2014). Hence CypD mediated MPTP inhibition could potentially be a valid treatment strategy for the treatment of AP secondary to excess alcohol intake.

5. Chapter 5 – Development and assessment of novel small molecule inhibitors of Cyclophilin D on pancreatic acinar cell function and cell death

5.1 Introduction

Cyclophilins have been highly conserved throughout evolution and are found in all prokaryotic and eukaryotic cells. All cyclophilins share a common domain of approximately 109 amino acids, the cyclophilin-like domain (CLD). The CLD is surrounded by domains which are unique to each member of the family and are associated with the subcellular compartmentalization and functional specialization (Marks 1996, Arevalo-Rodriguez, Wu et al. 2004). Cyclophilins act to stabilize the *cis-trans* transition state and accelerate isomerization, a process considered important in protein folding and assembling of multi-domain proteins (Gothel and Marahiel 1999).

Peptide bonds can exist in two distinct isomeric forms: *cis* and *trans.* The lower energy state *trans* peptide bonds have side chains 180 degrees opposite to each other are sterically favoured and ribosomes tend to synthesize peptide bonds in this form. Many proline containing proteins however have a preceding peptide bond (peptidyl-prolyl) in *cis* form with side chains adjacent to each other. *De novo* protein folding and the refolding processes involved in cellular membrane trafficking require isomerization of peptide bonds in *cis* form. The spontaneous isomerization of peptidyl-prolyl bonds requires energy, is a slow process and therefore a rate-limiting step in folding which is accelerated by the *cis-trans* isomerase activity (see figure 5.1) of cyclophilins (Gothel and Marahiel 1999).

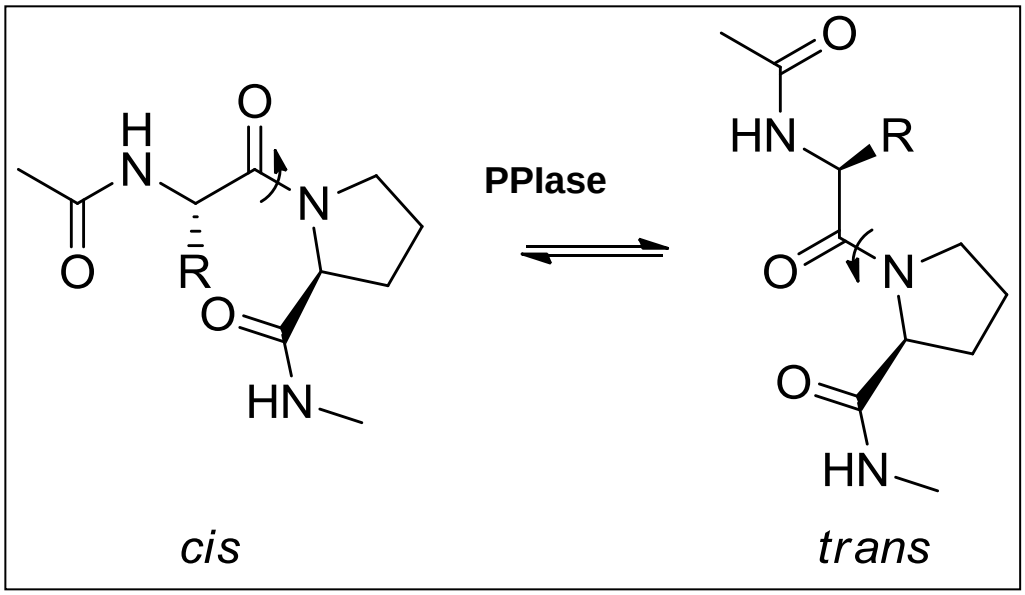


Figure 5.1 - Schematic illustration of the mechanism of cyclophilin action

The interconversion between *trans* and *cis* isomers of the peptide bond between proline and another amino acid is catalysed by cyclophilins acting as peptidyl-prolyl isomerases (PPIases). *Modified from (Shore, Awais et al. 2016)*

As previously mentioned CypD, a mitochondrial targeted PPIase, is a key modulator of MPTP opening. Since MPTP formation and subsequent mitochondrial dysfunction play a critical role in pancreatic necrosis as well as the pathophysiology of AP, CypD appears to be a valid target for the treatment of AP. There is further evidence to suggest that the characteristic local and systemic pathological responses are significantly reduced in CypD knockout mice (Elrod and Molkentin 2013, Mukherjee, Mareninova et al. 2015). Although CsA, a lipophilic cyclic peptide used as an immunosuppressant drug, has nanomolar binding affinity for cyclophilins (see figure 5.2), this interaction is non-specific as CsA binds to CypA, CypB and CypD (Gaither, Borawski et al. 2010, Hopkins and Gallay 2012). Interaction of CsA with cytosolic CypA generates a complex that has an ability to bind to, and inhibit calcineurin. As a consequence, the calcineurin substrate phospho-nuclear factor of activated T-cells is unable to translocate to the nucleus for initiation of an immune response (Liu, Farmer et al. 1991). Theoretically, this immunosuppressive effect could be counterproductive in AP as it may predispose sterile pancreatic necrosis to secondary infections. Interestingly, there is however evidence to suggest that high doses of CsA aggravate AP (Foitzik, Forgacs et al. 1998) and therapeutically recommended doses of CsA can lead to abnormal pancreatic enzyme secretion and induce AP under conditions of pancreatic stimulation by caerulein (Ito, Kimura et al. 1993). Prolonged exposure to CsA in combination with caerulein has also been shown to distort pancreatic repair, transforming caerulein induced pancreatitis into a fibrotic chronic-like disease. The mechanism involves TGF-beta, myofibroblasts, and defective collagenase activation (Vaquero, Molero et al. 1999).

Non-immunosuppressant semisynthetic analogues of CsA, such as DEB025 and NIM811 although maintain inhibition of cyclophilins but do not bind to calcineurin (Hopkins and Gallay 2012), have unfavourable drug-like characteristics with high molecular weights, limited solubility and poor bioavailability (Dunsmore, Malone et al. 2011). It would therefore appear logical to initiate a drug discovery programme to identify novel, small molecule inhibitors of CypD which would exhibit high selectivity for CypD over other cyclophilins, have improved pharmacokinetic / pharmacodynamic properties and do not have immunosuppressive effect. The aim of this aspect of the study is to identify and develop novel, small molecular inhibitors specific for CypD; characterise their effectiveness in inhibiting the MPTP and their efficacy in reducing activation of necrotic cell death pathway in PACs.

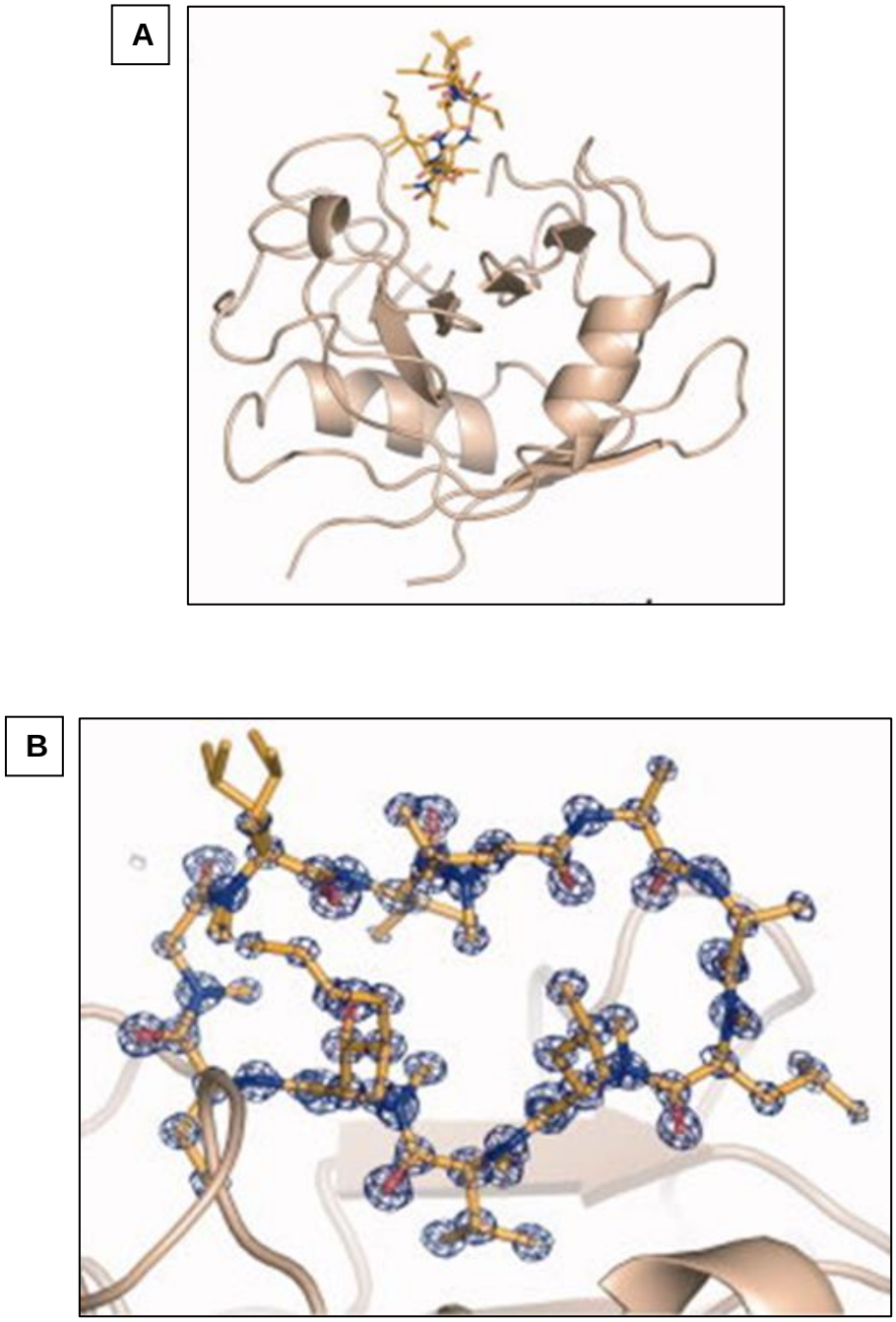


Figure 5.2 - Crystal structure of human CypD in complex with CsA

(A) Overall structure of CypD in complex with CsA. CypD is composed of eight β-strands, two α helices and one 3_{10} helix. CypD and CsA are shown as ribbon- and stick-models, respectively. (B) Close-up view of CsA superimposed on its Fo-Fc omit electron density map, shown as a blue mesh. *Adapted from (Kajitani, Fujihashi et al. 2008)*

5.2 Methods

Compounds predicted to bind strongly to CypD were identified using computational molecular modelling. A virtual screening database was used to select compounds that have properties similar to the active part of CsA which interacts with CypD. Firstly, molecules with “bad” sub structures and chiral compounds were excluded. Next, molecular modelling approach was used to examine protein ligand docking and identify compounds that are predicted to bind strongly to CypD (see figure 5.3). Recombinant CypD was produced as previously described (section 2.6) and binding of the screened low molecular weight CypD inhibitors was evaluated using a biophysical assay (surface plasmon resonance – section 2.7) to identify potential ‘hits’. The compounds selected were small molecular weight molecules, more likely to be membrane permeable and easily absorbed, that obeyed the Lipinski’s Rule of Five (Lipinski, Lombardo et al. 2001) which states that an orally active drug has no more than one violation of the following:

(i) No more than 5 hydrogen bond donors

(ii) No more than 10 hydrogen bond acceptors

(iii) Molecular weight of less than 500 daltons

(iv) Octanol-water partition co-efficient log P not more than 5

Candidates with high affinity for CypD were subsequently tested in live murine PACs using confocal microscopy to measure $\Delta\psi_m$ and activation of necrotic cell death pathway. Cells were exposed to TLCS in the presence and absence of the newly identified ‘hits’ to evaluate protection against $\Delta\psi_m$ and compounds which conferred mitochondrial protection were tested further in necrosis

assays. In order to evaluate the potential *in vitro* toxicity of the newly identified small molecular CypD inhibitors, PACs were incubated with the test compounds in the necrotic cell death assays. All compounds were purchased from Enamine Ltd (Ukraine) or synthesised by our collaborators in medicinal chemistry.

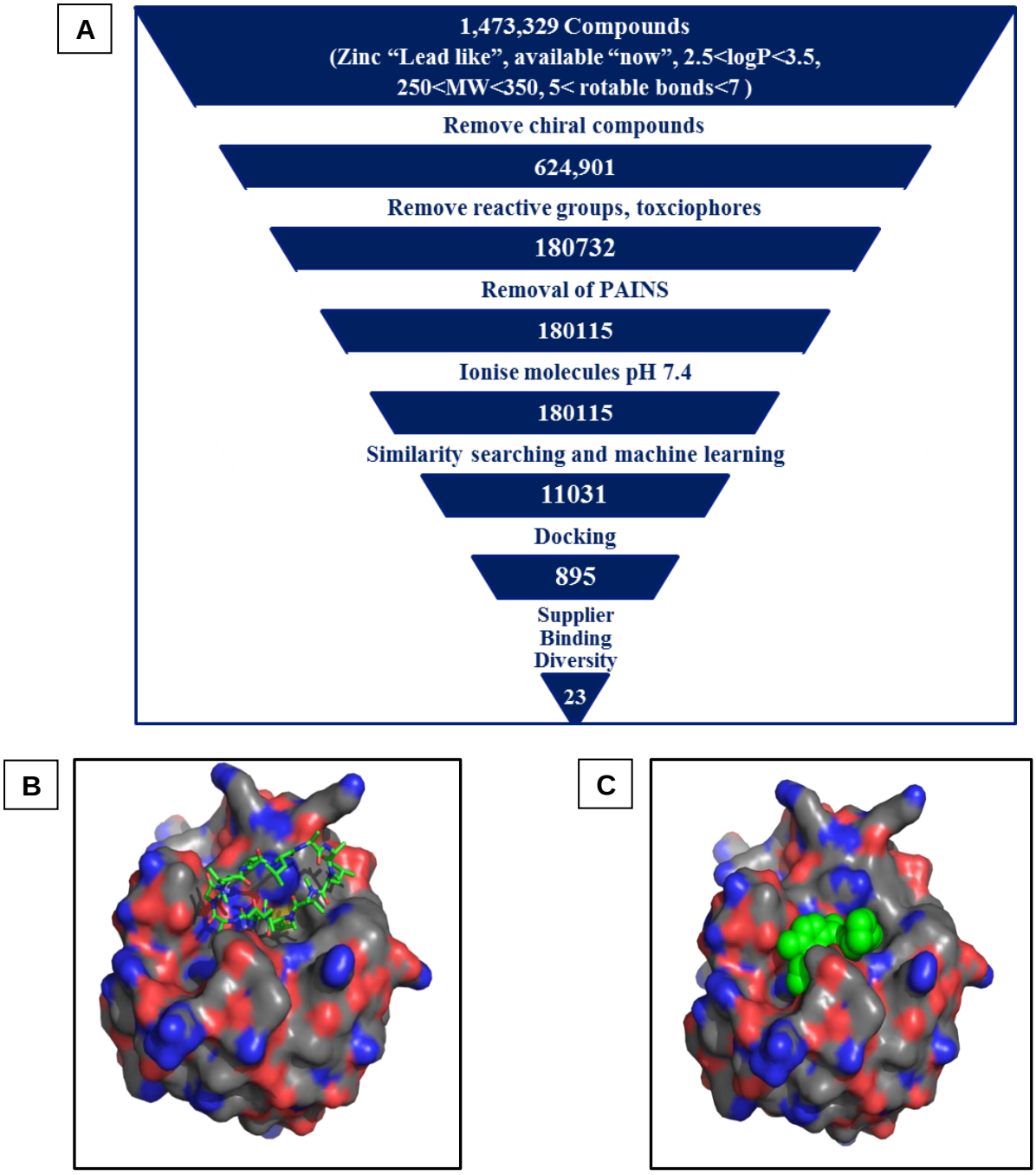


Figure 5.3 - Virtual screening for selecting novel small molecular CypD inhibitors

(A) Firstly, molecules with "bad" sub structures and chiral compounds were excluded. Next, molecular modelling approach was used to examine protein ligand docking and identify compounds that were predicted to bind strongly to CypD. (B) Molecular modelling showing binding site of CsA on human CypD and (C) newly identified small molecule CypD inhibitor binding to CypD in same region as CsA. *Courtesy Neil Berry*

5.3 Results

Evaluation of small molecule CypD inhibitors; binding with CypD, effects on mitochondrial function and necrotic cell death

Twenty- three compounds were predicted to bind with CypD using the virtual screening protocol and subsequently evaluated using SPR assay as previously described. The results of the biophysical SPR assay identified six compounds namely; 4256, 9094, 3326, 6256, EA4 and AP-1A02 that showed binding affinity with mutant CypD. Pre-treatment with compounds 4256 (see figure 5.5) and 9094 (see figure 5.7) prevented loss of $\Delta\psi_m$ in response to 500 µM TLCS, however showed little effect on PI uptake. Compound 4256 displayed toxicity on its own in the cell death assay. Compounds 3326 (see figure 5.9) and 6256 (see figure 5.11) showed little or no mitochondrial protection in response to 500 µM TLCS and were therefore not tested in cell death assay. Compounds EA4 and E6 were synthesised by our collaborators in medicinal chemistry. Pre-treatment with compound EA4 at a dose of 10 µM prevented TLCS induced loss of $\Delta\psi_m$ and reduced necrosis (see figure 5.12) and compound E6, which did not show any binding with CypD in SPR, was tested as an internal control and did not prevent bile acid induced mitochondrial depolarisation or necrotic cell death (see figure 5.13) as predicted. Out of 23 compounds screened by SPR, one lead compound named AP-1A02 was identified (see figure 5.14 for chemical structure and molecular docking). AP-1A02 showed strong binding with Cyp D (Kd = 0.8 µM) and inhibited its enzymatic activity (Ki = 1.7 µM) as shown in figure 5.15. AP-1A02 protected $\Delta\psi_m$, prevented necrotic cell death

pathway activation of freshly isolated murine as well as human PACs at a dose of 5 µM (see figure 5.16).

5.3.1 Evaluation of small molecule CypD inhibitor - compound 4256; binding with CypD, effects on mitochondrial function and necrotic cell death

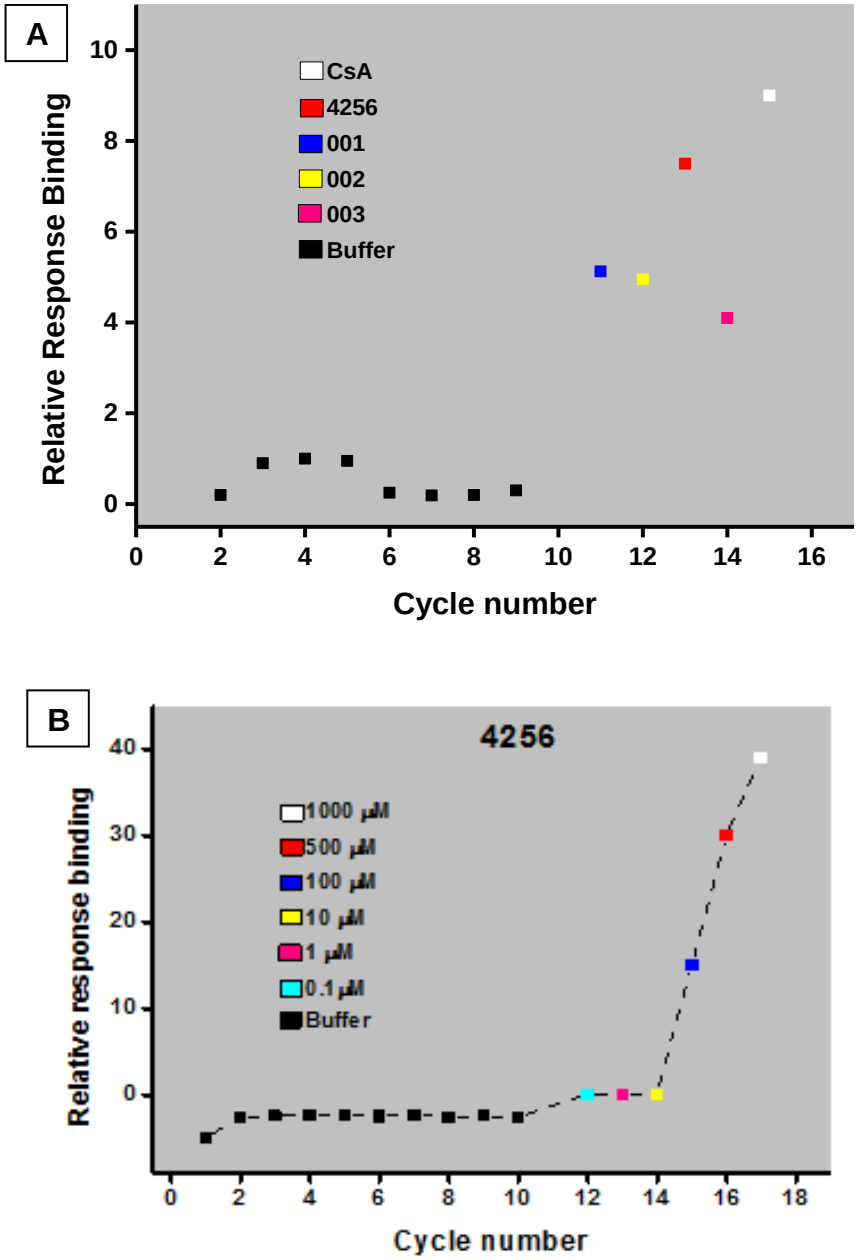


Figure 5.4 - SPR analysis of binding of compound 4256 to CypD

Screening was performed by adding 100µM of each compound over recombinant CypD immobilized on a CM5 sensor chip and CsA used as a positive control. Compound 4256 showed strong binding with CypD compared to other compounds (A). Concentration dependent binding of 4256 with CypD showed highest binding at 1 mM (B), the dose at which this compound was tested in subsequent cell based assays.

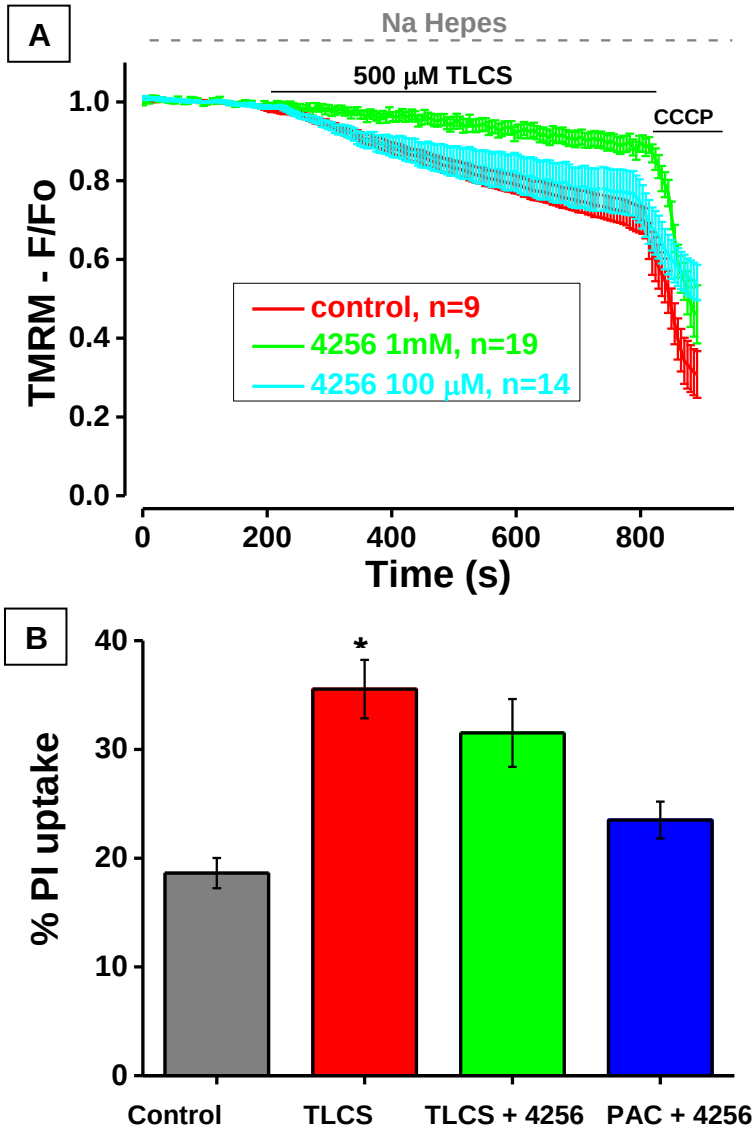


Figure 5.5 - Pathologic PAC $\Delta\psi_m$ and necrotic cell death responses to TLCS, in the presence or absence of 4256

$\Delta\psi_m$ changes in response to TLCS (500 µM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with 4256 (1mM) decreased the level of depolarisation (green trace), whereas at a dose of 100 µM failed to preserve $\Delta\psi_m$ (yellow trace). Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) $\pm$ SEM, with respective number of recordings shown in the key (A). Compound 4256 (1mM) did not protect murine cells from plasma membrane disruption and on its own increased PI uptake. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (B).

5.3.2 Evaluation of small molecule CypD inhibitor - compound 9094; binding with CypD, effects on mitochondrial function and necrotic cell death

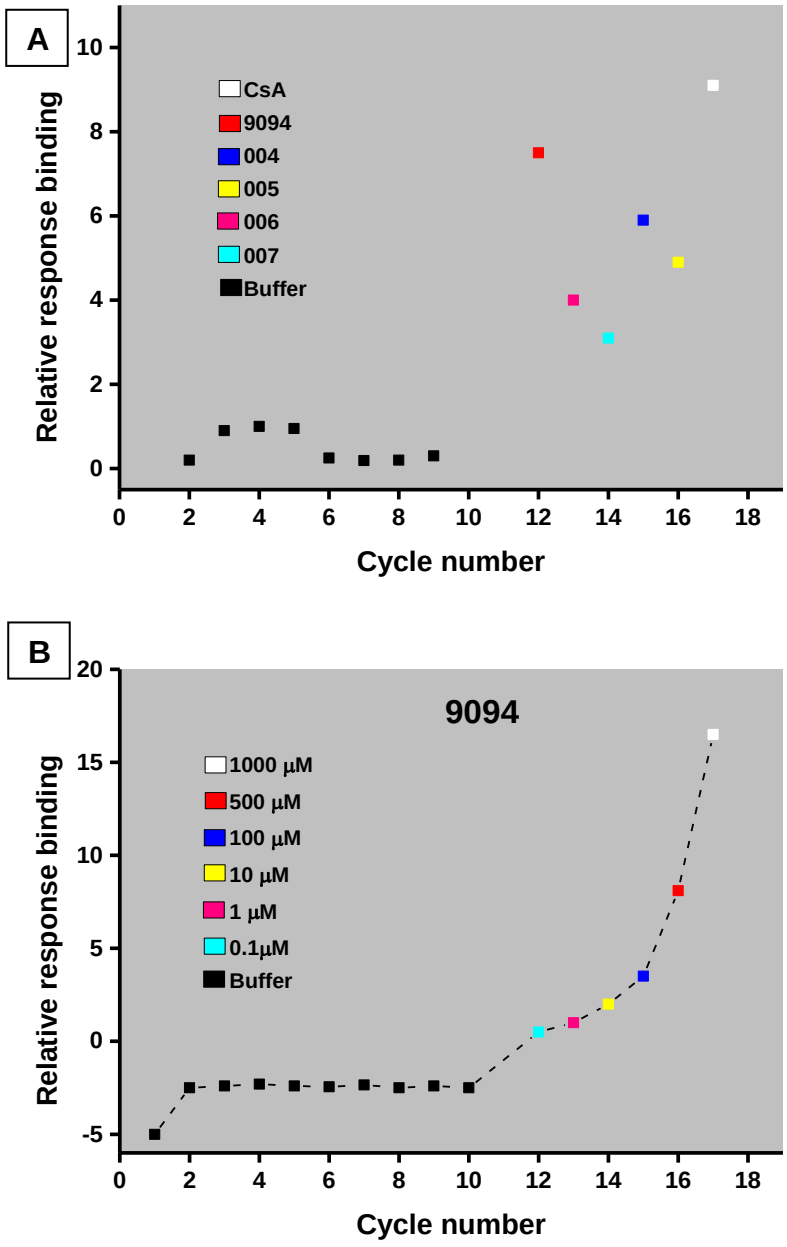


Figure 5.6 - SPR analysis of binding of compound 9094 to CypD

Screening was performed by adding 100µM of each compound over recombinant CypD immobilized on a CM5 sensor chip and CsA used as a positive control. Compound 9094 showed strong binding with CypD compared to other compounds (A). Concentration dependent binding of 9094 with CypD showed highest binding at 1 mM (B) and significant binding at 500 µM, therefore tested at lower dose (500 µM) in subsequent cell based assays.

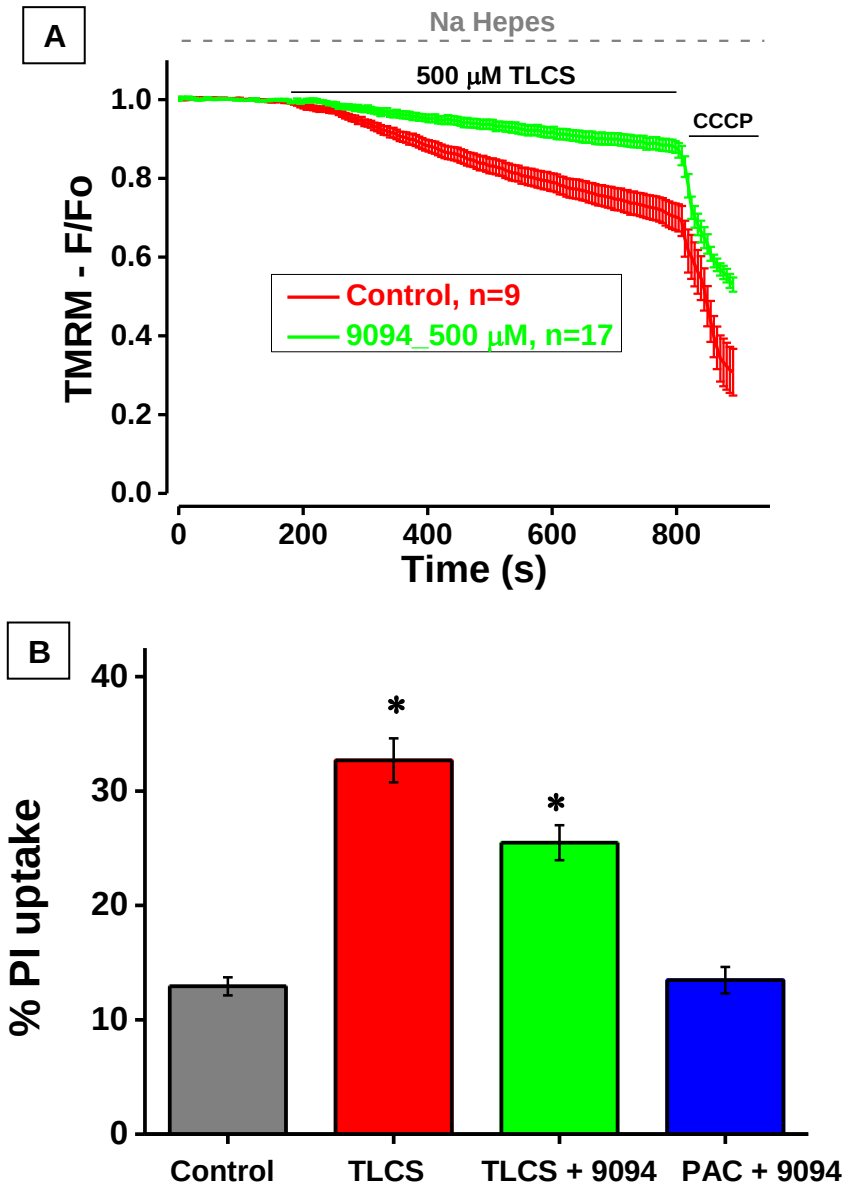


Figure 5.7 - Pathologic PAC $\Delta\psi_m$ and necrotic cell death responses to TLCS, in the presence or absence of 9094

$\Delta\psi_m$ changes in response to TLCS (500 µM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with 9094 (500 µM) decreased the level of depolarisation (green trace). Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key (A). Compound 9094 (500 µM) partially protected murine cells from plasma membrane disruption and on its own did not increase PI uptake. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (B).

5.3.3 Evaluation of small molecule CypD inhibitor - compound 3326; binding with CypD and effects on mitochondrial function

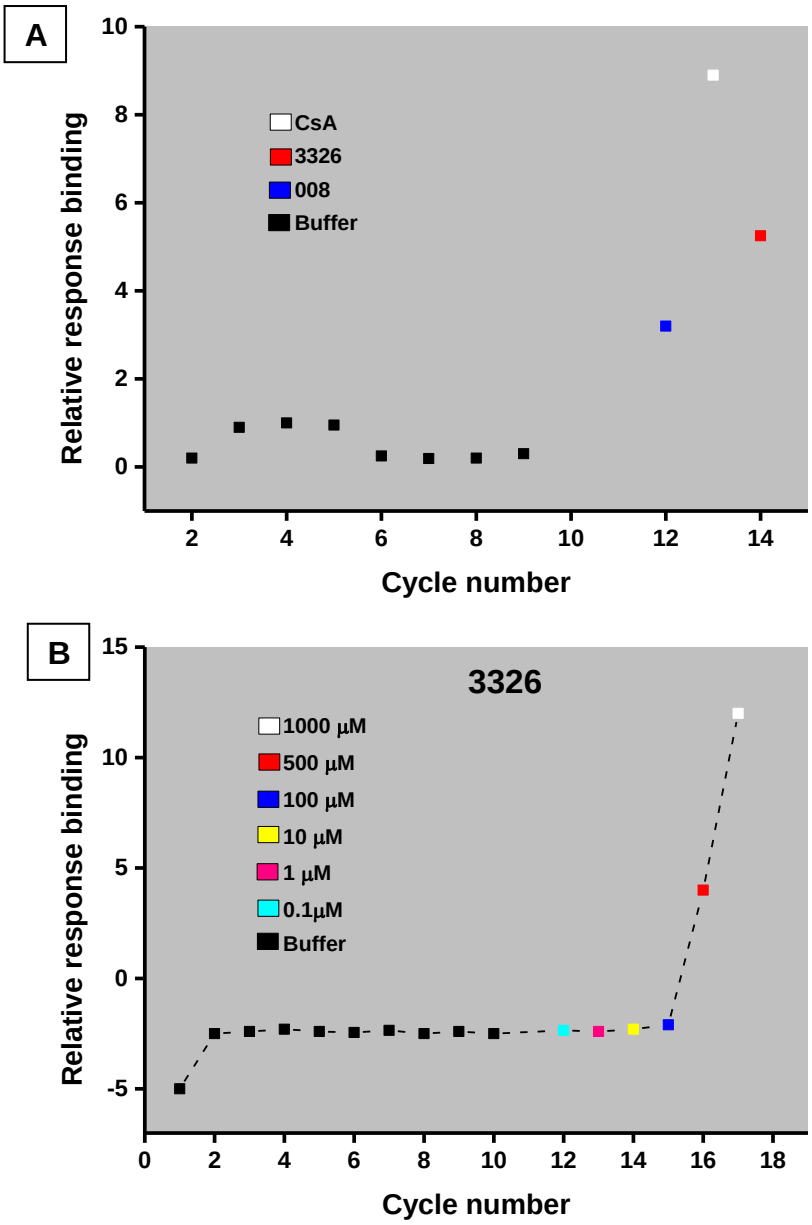


Figure 5.8 - SPR analysis of binding of compound 3326 to CypD

Screening was performed by adding 100µM of each compound over recombinant CypD immobilized on a CM5 sensor chip and CsA used as a positive control. Compound 3326 showed strong binding with CypD compared to other compounds (A). Concentration dependent binding of 3326 with CypD showed highest binding at 1 mM (B) and significant binding at 500 µM, therefore tested at lower dose (500 µM) in subsequent cell based assays.

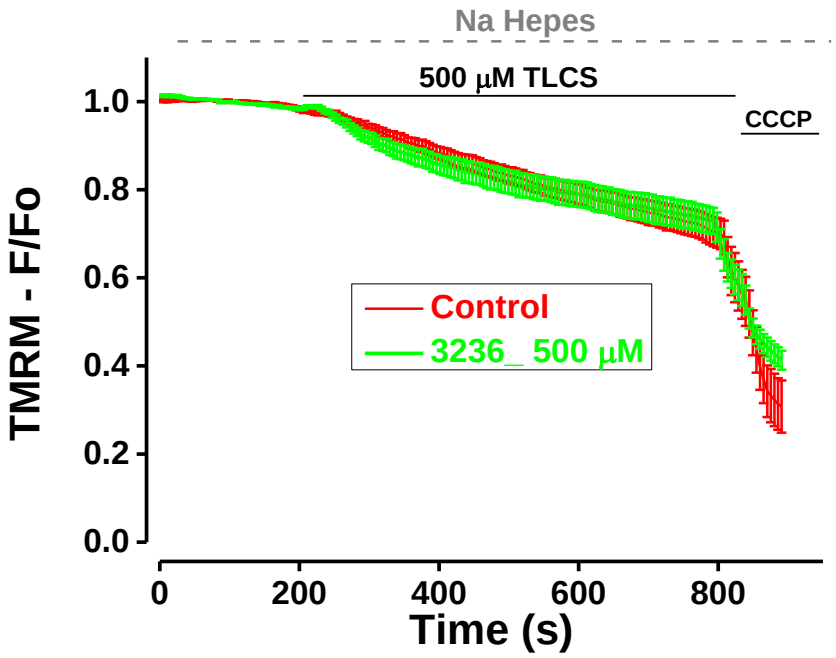


Figure 5.9 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of 3326

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with 3326 (500 μM) had no significant effect on the level of depolarisation (green trace) and was therefore not tested in necrosis assays. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) $\pm$ SEM, with respective number of recordings shown in the key.

5.3.4 Evaluation of small molecule CypD inhibitor - compound 6256; binding with CypD and effects on mitochondrial function

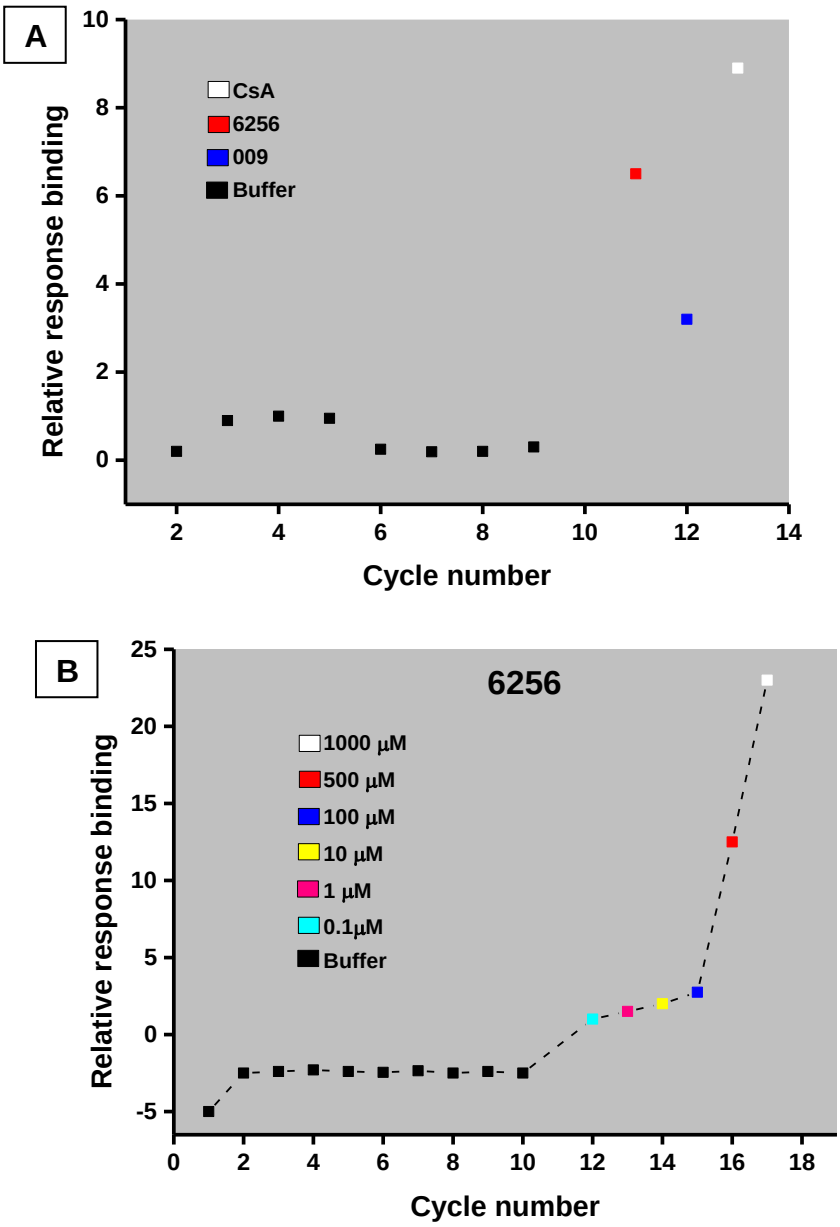


Figure 5.10 - SPR analysis of binding of compound 6256 to CypD

Screening was performed by adding 100µM of each compound over recombinant CypD immobilized on a CM5 sensor chip and CsA used as a positive control. Compound 6256 showed strong binding with CypD compared to other compounds (A). Concentration dependent binding of 6256 with CypD showed highest binding at 1 mM (B) and significant binding at 500 µM, therefore tested at lower dose (500 µM) in subsequent cell based assays.

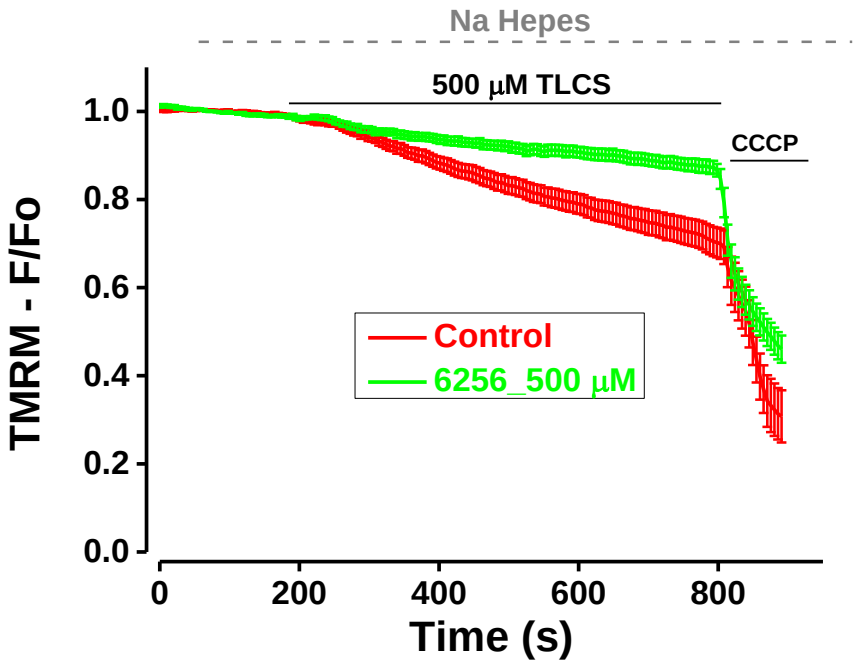


Figure 5.11 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of 6256

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with 6256 (500 μM) had partial protective effect on the level of depolarisation (green trace) and was therefore not tested in necrosis assays. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

5.3.5 Evaluation of small molecule CypD inhibitor - compound EA-4; effects on mitochondrial function and necrotic cell death

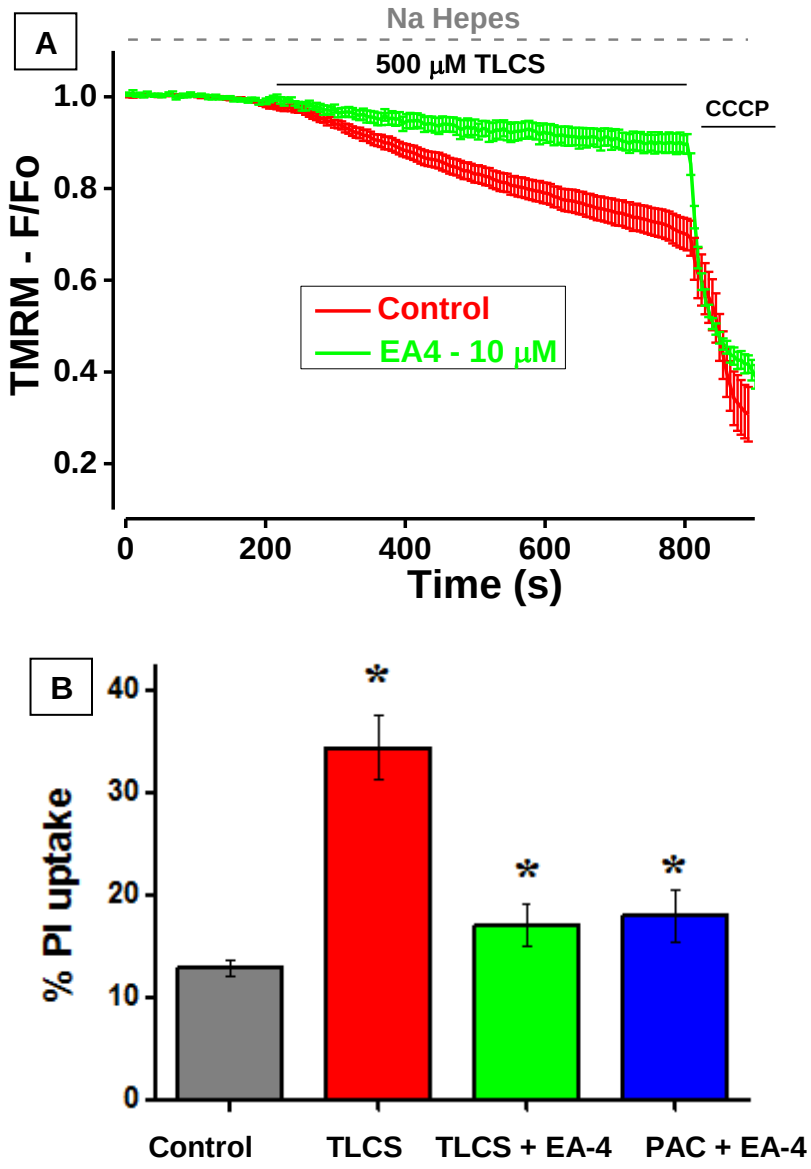


Figure 5.12 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of EA-4

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with EA-4 (10 μM) decreased the level of depolarisation (green trace). Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key (A). Compound EA-4 (10 μM) partially protected murine cells from plasma membrane disruption and on its own did not increase PI uptake. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (B).

5.3.6 Evaluation of small molecule CypD inhibitor - compound E6; effects on mitochondrial function and cell death

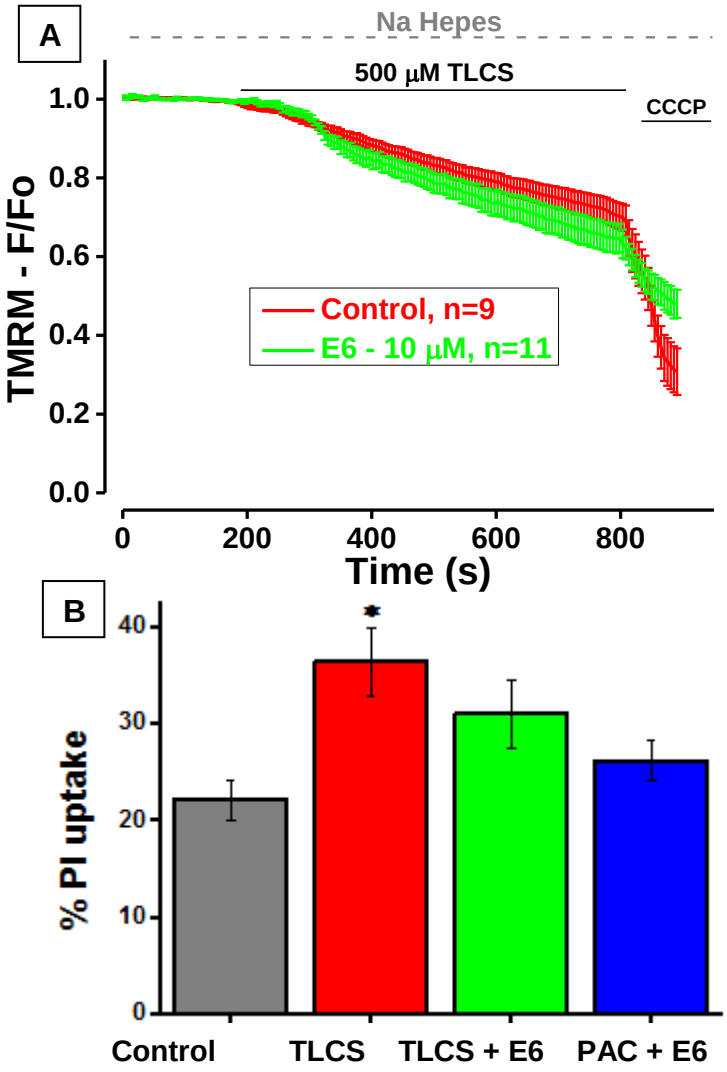


Figure 5.13 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of E6

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with E6 (10 μM) had no effect on level of depolarisation (green trace). Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key (A). Compound E6 (10 μM) failed to protect murine cells from plasma membrane disruption and on its own slightly increased PI uptake. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (B). The experiment was completed to demonstrate concordance between biological and biophysical assays in case a compound does not bind CypD on SPR.

5.3.7 Evaluation of small molecule CypD inhibitor - compound AP-1A02; binding with CypD, effects on mitochondrial function and cell death

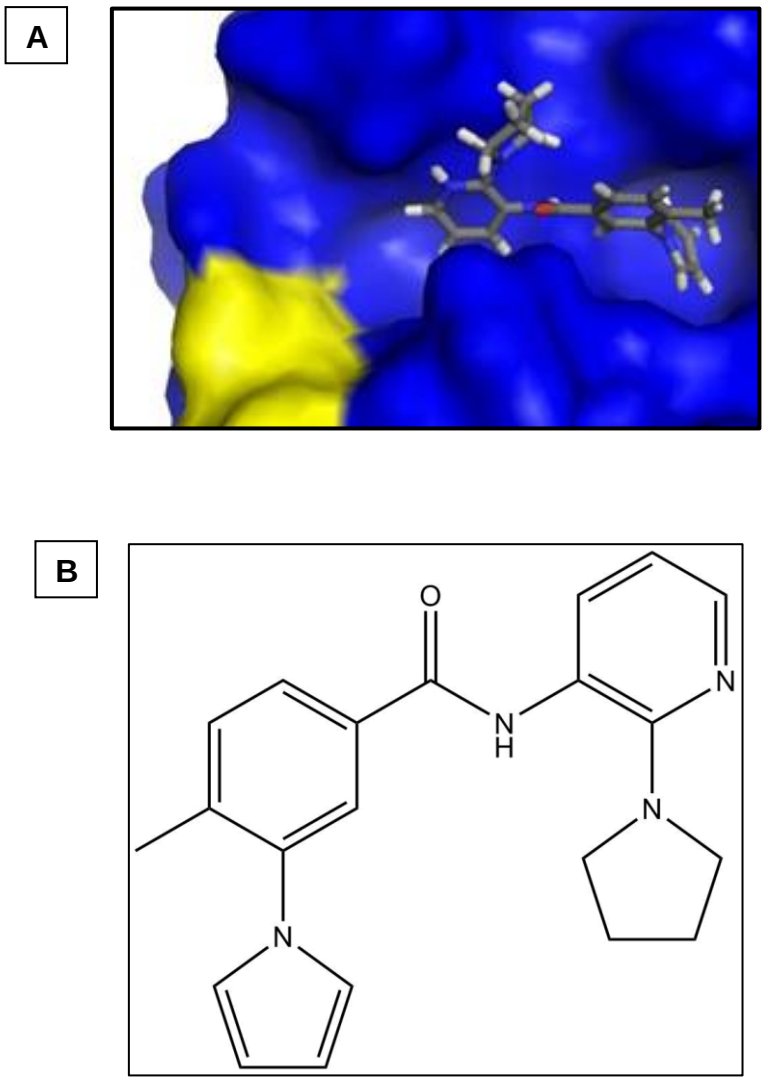


Figure 5.14 - Molecular docking and chemical structure of lead compound AP-1A02

(A) Molecular modelling showing binding site of AP-1A02 on human CypD in same region as CsA *(courtesy Neil Berry).* (B) Chemical structure of AP-1A02.

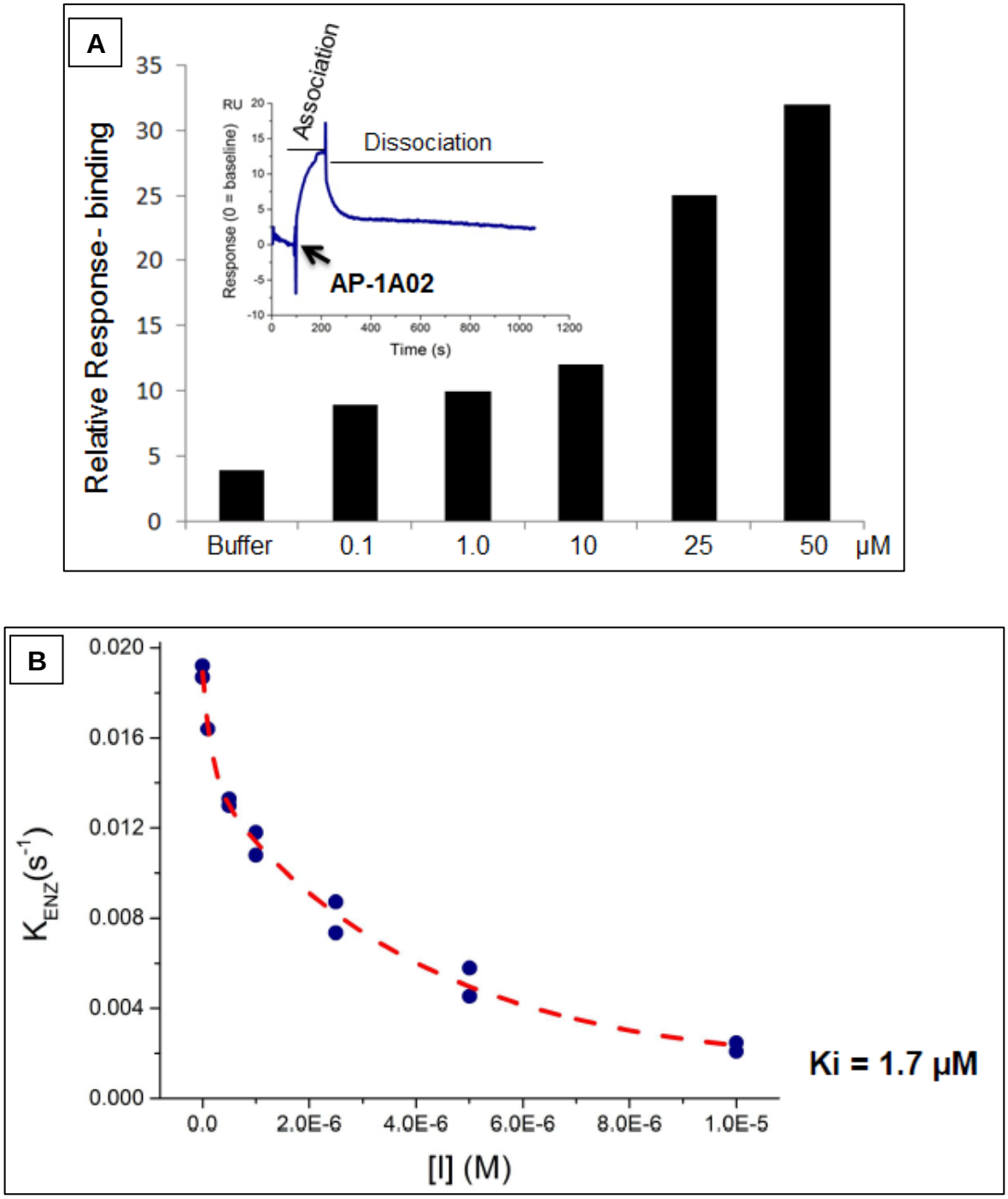
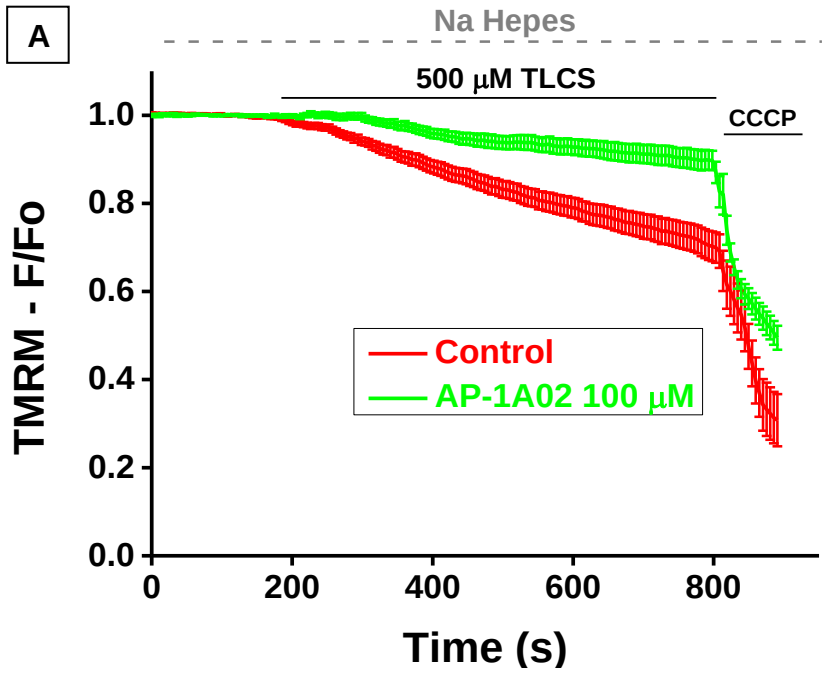
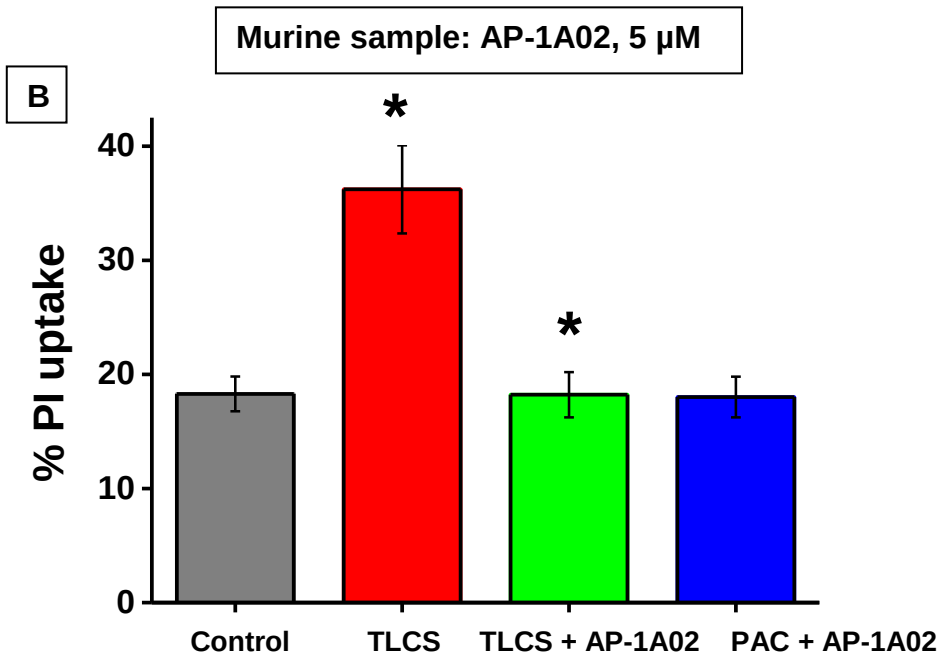


Figure 5.15 - Dose dependent response of AP-1A02 on SPR

Assay performed by passing AP-1A02 over recombinant CypD immobilized on a CM5 sensor chip. Inset, kinetics of AP-1A02 (10 μM). K_d of AP-1A02 was calculated by dividing the rate of association by the rate of association (A). AP-1A02 inhibits PPIase activity of CypD. N-SUCCINYL-ALA-ALA-PRO-PHE-P-NITROANILIDE was used as a substrate. A first order rate was fitted to the absorbance (330 nm) data to obtain a rate constant. The catalytic rate was calculated from the enzymatic rate minus the background rate (B).


A
Na Hepes
500 µM TLCS
CCCP
TMRM - F/Fo
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
Time (s)
Control
AP-1A02 100 µM


Murine sample: AP-1A02, 5 µM
B
% PI uptake
40
30
20
10
0
*
*
Control
TLCS
TLCS + AP-1A02
PAC + AP-1A02

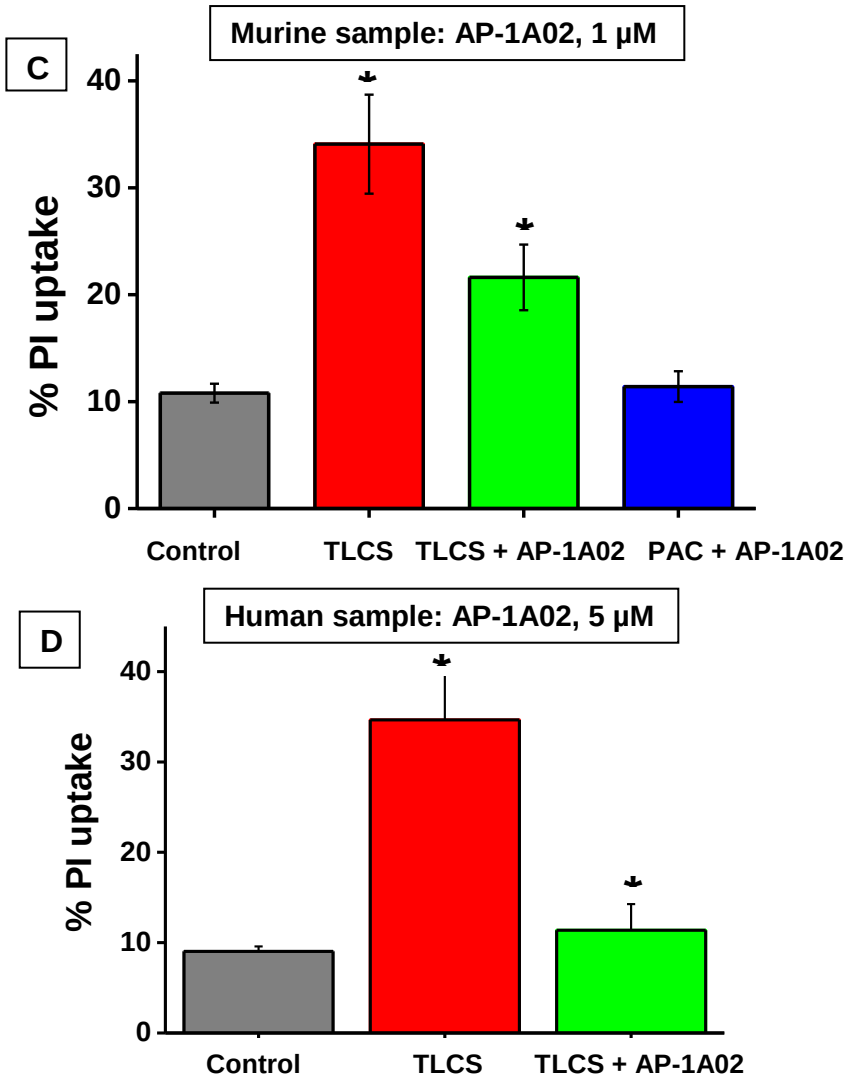


Figure 5.16 - Pathologic PAC $\Delta\psi_m$ and necrotic cell death responses to TLCS, in the presence or absence of AP-1A02

$\Delta\psi_m$ changes in response to TLCS (500 µM) show significant depolarisation (red trace) with complete mitochondrial depolarisation on application of protonophore, CCCP. Pre-treatment with AP-1A02 (100 µM) decreased the level of depolarisation (green trace). Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key (A). Compound AP-1A02 5µM (B) and 1 µM (C) protected murine cells from plasma membrane disruption and on its own had no effect on PI uptake. Each bar is mean ± SEM of 16 fields of view from each of a minimum of three mice (B and C). Compound AP-1A02 5µM tested in one human sample and protected human PACs from activation of necrotic cell death pathway.

5.4 Discussion

A drug discovery programme is initiated when there is unmet clinical need for a disease or clinical condition for which there is lack of availability of appropriate medical products. Given the dearth of targeted therapy for AP, a common and potentially life threating disease, it is but logical to invest in a robust drug discovery programme for identification of new targets and development of new compounds that could be used for the treatment of pancreatitis.

There are two different approaches to drug discovery, target-based screening and phenotypic screening. With substantial experimental evidence to support that mitochondrial CypD is a promising molecular target for treatment of necrotizing pancreatitis (Mukherjee, Mareninova et al. 2015), the target-based methodology has been adopted to identify CypD inhibitors in this study. This strategy allows for the application of molecular and chemical knowledge to investigate specific molecular hypotheses and utilise small-molecule screening strategies, the advantages of which have previously been discussed.

The lead compound, AP-1A02, identified as a result of this strategy is a novel, small molecule CypD inhibitor with a molecular weight of 246. The first compound that was identified as part of the screening process showed biological efficacy at 1mM dose whereas AP-1A02 has significantly reduced bile acid induced necrotic cell death not only in murine but also in human PACs at a dose of 5µM. Testing compounds selectively on freshly isolated human PACs reduces the chances of failure of a compound at a later stage in the drug discovery process. AP-1A02 also did not demonstrate any toxicity on its own,

unlike other compounds which showed an increase in necrotic cell death when incubated with PACs. This compound can form the basis of a lead optimization chemistry programme to increase potency of the chemical series. AP-1A02 could not be tested *in vivo*, due to lack of solubility in the desired solvent.

The next steps would involve collaboration with medicinal chemistry to improve the water solubility and efficacy in the nanomolar range and undertake assays such as; X-ray crystallography - to obtain high-resolution structures of the complexes, isothermal titration calorimetry - to derive the thermodynamic signature of the interactions and PPIase assay - to determine the cis/trans isomerisation kinetics and specificity. These assays can be crucial in optimizing lead molecules. Cyclophilin family members have high sequence homology, and therefore it is a challenge to develop an inhibitor specific to CypD (Davis, Walker et al. 2010). In order to set up a counter-screening programme, recombinant CypA and CypB will need to be produced to assess the binding affinity of these newly identified small molecular CypD inhibitors with CypA and CypB. Structural data would also be useful to optimize inhibitor(s) specific for CypD to prevent off target effects. Prior to submitting small molecules for evaluation in an *in vivo* model of AP, further medicinal chemistry modifications will have to be untaken to optimise solubility, metabolic stability, plasma protein binding and where appropriate permeability of drug leads. Pharmacokinetic studies in the mouse can then be performed to ensure lead molecules have acceptable exposure following oral doses of drug and these can be tested in experimental models of AP to evaluate their efficacy and safety profiles.

Other approaches for identification of new small molecular inhibitors of CypD would include the use of 2D quantitative structure activity relationship (QSAR) alongside 3D ligand-based pharmacophore models as described by Valasani et al (Valasani, Vangavaragu et al. 2014). Compounds screened and identified using this strategy can be used for molecular docking to interpret the efficacy of the novel molecules in inhibiting the enzymatic activity of CypD. Another strategy that can be employed to use compounds previously identified as potent inhibitors of cyclophilins (Guichou, Viaud et al. 2006, Li, Chen et al. 2006, Ni, Yuan et al. 2009) as a backbone to synthesize a series of molecules and characterise their ability to inhibit CypD.

It must also be highlighted that CypD inhibitors have a much wider clinical utility as preventing MPTP formation via CypD inhibition is also implicated in several other diseases such as ; cardiac and cerebral ischaemic-reperfusion injury (Fakharnia, Khodagholi et al. 2017, Javadov, Jang et al. 2017), neurodegeneration in multiple sclerosis (Warne, Pryce et al. 2016), Alzheimer's disease (Du, Guo et al. 2008), counteracting skeletal muscle dysfunction in mitochondrial myopathy (Gineste, Hernandez et al. 2015), Duchenne muscular dystrophy (Reutenauer, Dorchies et al. 2008), development of acute lung injury following endotoxaemia (Fonai, Priber et al. 2015), liver cancer (Chen, Feng et al. 2012), beta-cell survival (Fujimoto, Chen et al. 2010) and ischaemic renal injury (Devalaraja-Narashimha, Diener et al. 2009) and hence this drug development programme has much wider implications.

6. Chapter 6 – Pharmacological inhibition of MPTP using TRO40303 - effects on pancreatic acinar cell function and experimental acute pancreatitis

6.1 Introduction

TRO40303 (3, 5-Seco-4-nor-cholestan-5-one oxime-3-ol) is a cholesterol-oxime cytoprotective compound developed by Trophos (now acquired by Roche) that binds to the outer mitochondrial translocator protein (TSPO), delaying MPTP opening and cell death in rat cardiomyocytes and has been shown to significantly reduce the extent of myocardial infarction in a rat model (Schaller, Paradis et al. 2010). TRO40303 has also been shown to protect HepG2 cells and primary mouse embryonic hepatocytes from palmitate intoxication and significantly reduce mortality in murine Fas-induced hepatotoxicity (Schaller, Michaud et al. 2015). Results from a randomized safety and tolerance phase 1 trial showed that TRO40303 can be safely administered intravenously in humans at doses expected to be pharmacologically active (Le Lamer, Paradis et al. 2014). MPTP inhibition using TRO40303, a drug quite far in clinical development has not been investigated before in the setting of AP. This chapter describes experiments designed to test the protective effects of TRO40303 on acinar cell function, cell death in response to pancreatic toxins and severity of EAP.

6.2 Methods

Three doses of TRO40303 - 1 µM(T1), 3 µM(T3) and 10µM(T10) were initially tested in live murine PACs using confocal microscopy to measure $\Delta\psi_m$, cytosolic calcium and activation of necrotic cell death pathway. Typically cells were exposed to bile acid - tauro-lithocholate sulphate (TLCS), cholecystokinin (CCK) and palmitoleic acid ethyl ester (POAEE) in the presence and absence

of TRO40303 to evaluate protection against $\Delta\psi_m$ loss and necrosis. All experiments were carried out with a constant concentration of DMSO (0.1%; Sigma, Gillingham, UK).T10 was also evaluated in protection against TLCS induced necrosis in human PACs. A liposomal preparation of TRO40303 (Batch TRO-003J 20 mg/ml) was subsequently tested therapeutically in three different models of murine EAP: hyperstimulation - caerulein induced experimental model (CER-AP), bile acid-induced murine acute pancreatitis (TLCS-AP) and alcoholic (ethanol and palmitoleic acid) pancreatitis (FAEE-AP).

6.3 Results

6.3.1 Acinar cell responses to TLCS in the presence of TRO40303

6.3.1.1 Mitochondrial and Ca^{2+} responses in the presence of 10μM TRO40303 (T10)

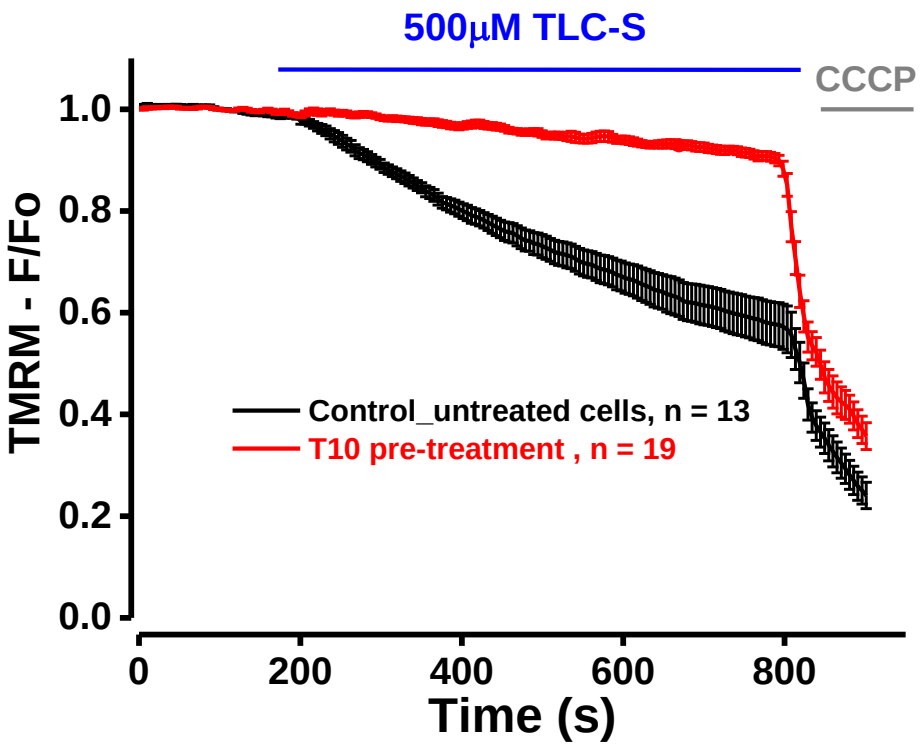


Figure 6.1 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of 10 μM TRO40303 (T10)

$\Delta\psi_m$ changes in response to TLCS (500 μM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T10 decreased the level of depolarisation, preserving $\Delta\psi_m$. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

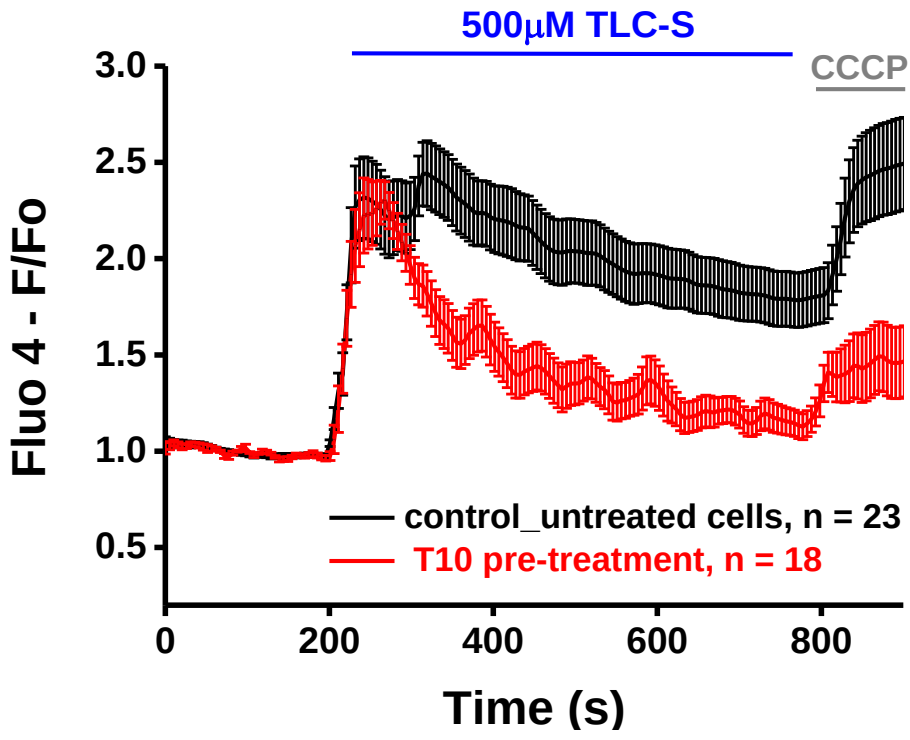


Figure 6.2 - Pathologic PAC Ca^{2+} responses to TLCS, in the presence or absence of 10 µM TRO40303 (T10)

Ca^{2+} changes in response to TLCS (500 µM) show typical large, rapid rise followed by a sustained plateau. Pre-treatment with T10 did not affect the initial rise in rise in but dampened the plateau phase. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.1.2 *Mitochondrial and Ca^{2+} responses in the presence of 3 µM TRO40303 (T3)*

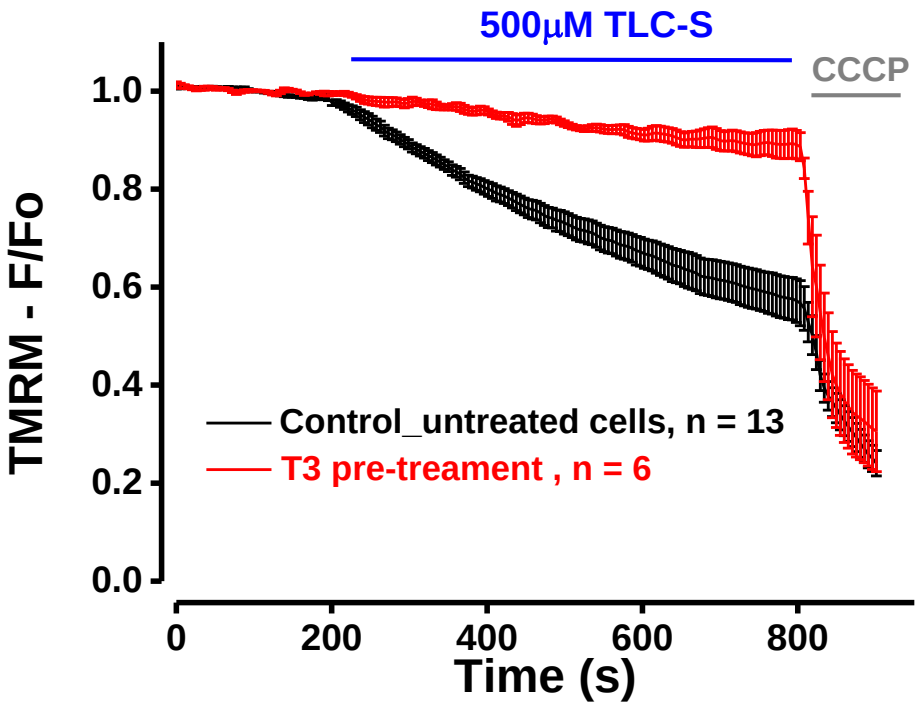


Figure 6.3 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of 3 µM TRO40303 (T3)

$\Delta\psi_m$ changes in response to TLCS (500 µM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T3 decreased the level of depolarisation, preserving $\Delta\psi_m$. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) $\pm$ SEM, with respective number of recordings shown in the key.

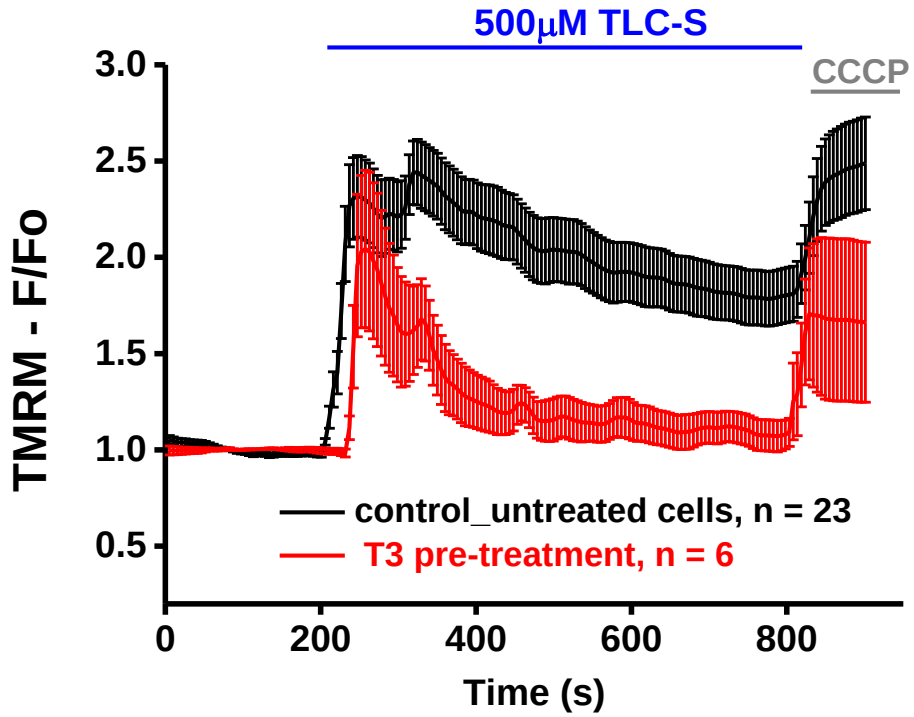


Figure 6.4 - Pathologic PAC Ca^{2+} responses to TLCS, in the presence or absence of 3 μM TRO40303 (T3)

Ca^{2+} changes in response to TLCS (500 μM) show typical large, rapid rise followed by a sustained plateau. Pre-treatment with T3 did not affect the initial rise in rise in but dampened the plateau phase. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.1.3 *Mitochondrial and Ca^{2+} responses in the presence of 1 µM TRO40303 (T1)*

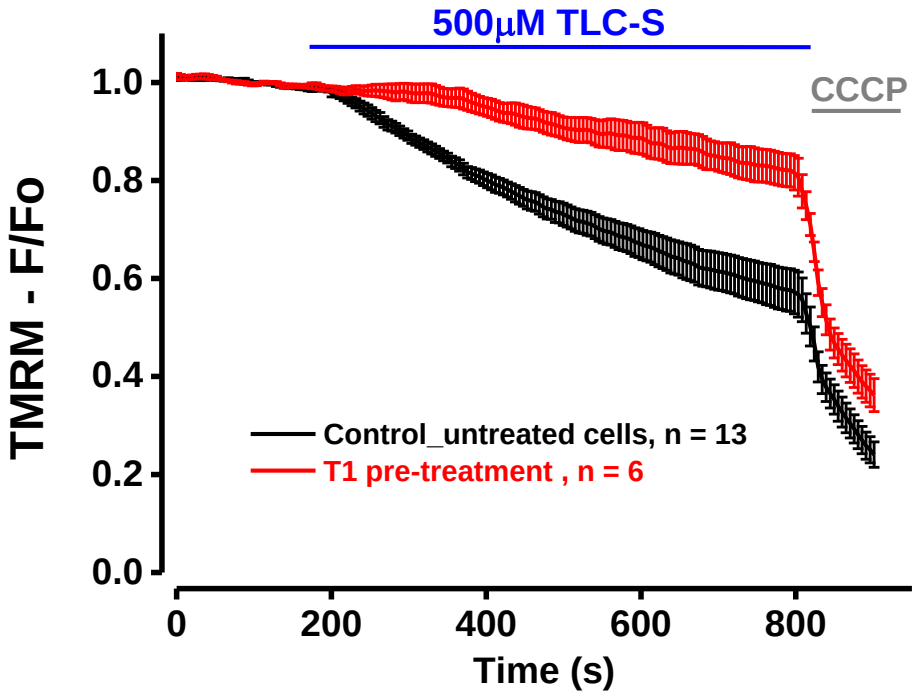


Figure 6.5 - Pathologic PAC $\Delta\psi_m$ responses to TLCS, in the presence or absence of 1 µM TRO40303 (T1)

$\Delta\psi_m$ changes in response to TLCS (500 µM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T1 decreased the level of depolarisation, preserving $\Delta\psi_m$. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

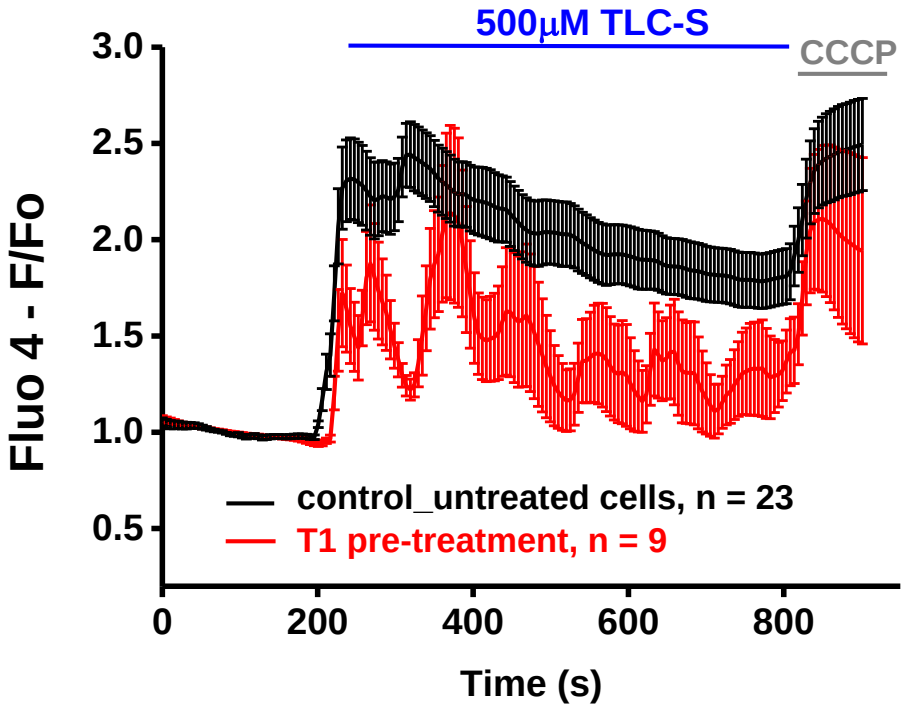


Figure 6.6 - Pathologic PAC Ca^{2+} responses to TLCS, in the presence or absence of 1 µM TRO40303 (T1)

Ca^{2+} changes in response to TLCS (500 µM) show typical large, rapid rise followed by a sustained plateau. Pre-treatment with T1 did not affect the initial rise in rise in but dampened the plateau phase. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.1.4 *Effects of TRO40303 on necrotic cell death pathway activation in response to TLCS in murine pancreatic acinar cells*

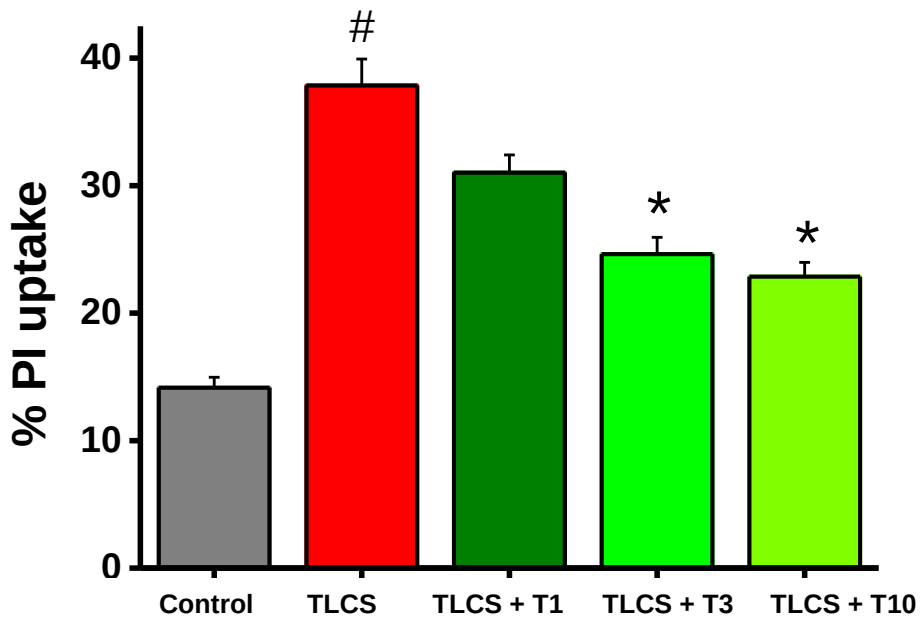


Figure 6.7 - PI uptake in response to TLCS in the presence or absence of TRO40303 in murine PACs

Co-administration of T3 and T10 with TLCS (500 µM) protected cells from early plasma membrane rupture (PI uptake) compared to untreated cells. Dose dependent effects of TRO40303 on necrotic cell death pathway activation can also be seen, *P<0.05, unpaired t- test.

6.3.1.5 *Effects of TRO40303 on necrotic cell death pathway activation in response to TLCS in human pancreatic acinar cells*

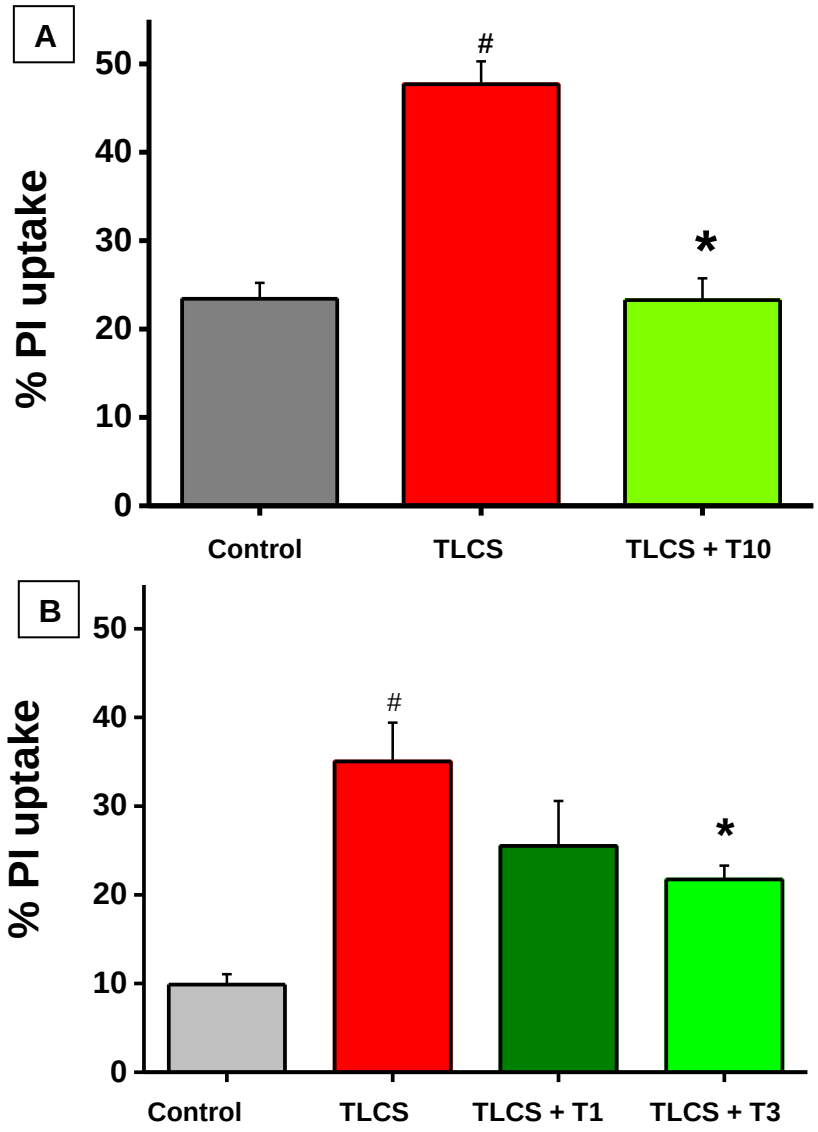


Figure 6.8 - PI uptake in response to TLCS in the presence or absence of TRO40303 in human PACs

Co- administration of T10 (A) and T3 (B) with TLCS (500 µM) protected cells from early plasma membrane rupture (PI uptake) compared to untreated cells. Dose dependent effects of TRO40303 on necrotic cell death pathway activation in human pancreatic acinar cells can also be seen, *P<0.05, unpaired t- test.

Note: *Results for human PACs are displayed in separate graphs; A) shows results using T10 tested in three human samples and B) shows results with T1& T3, which were tested in two separate human samples, hence the differences in control levels of cell death and response to TLCS in the two graphs.*

6.3.2 Acinar cell responses to CCK in the presence of TRO40303

6.3.2.1 *Mitochondrial and Ca^{2+} responses in the presence of 10 µM TRO40303*

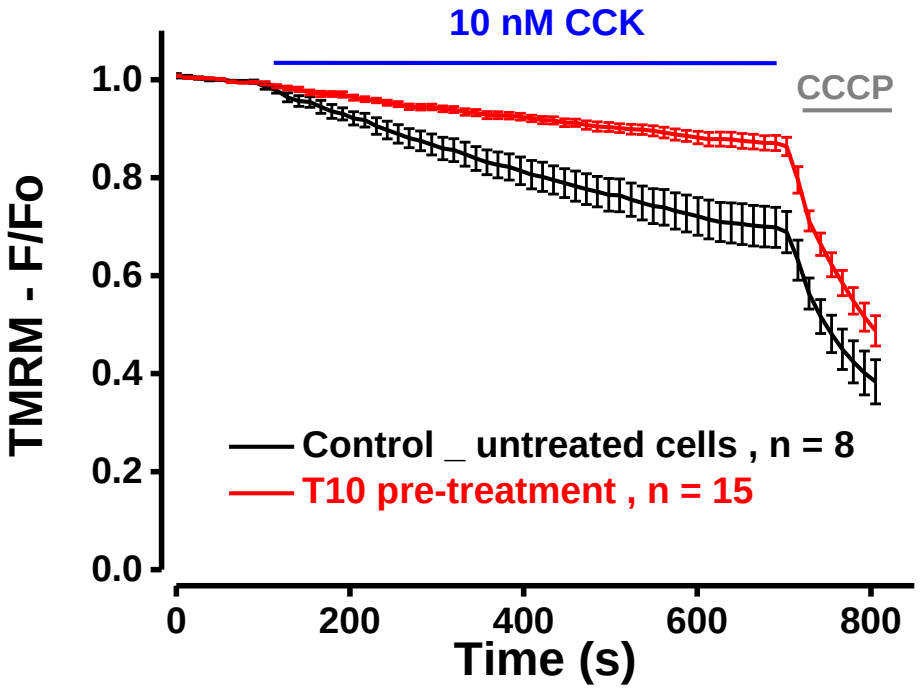


Figure 6.9 - Pathologic PAC $\Delta\psi_m$ responses to CCK, in the presence or absence of 10 µM TRO40303 (T10)

$\Delta\psi_m$ changes in response to CCK (10 nM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T10 decreased the level of depolarisation, preserving $\Delta\psi_m$. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

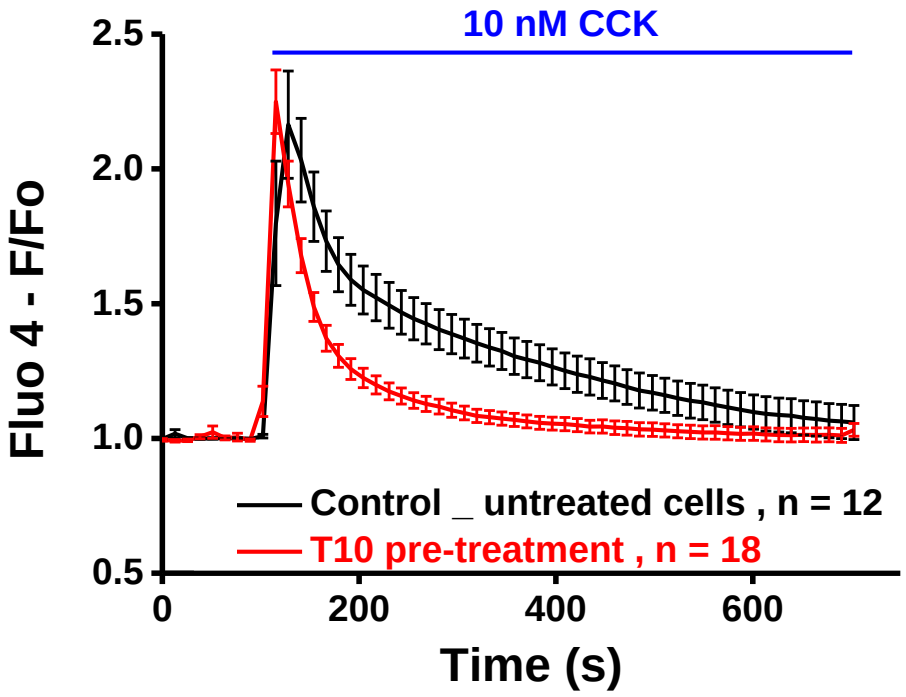


Figure 6.10 - Pathologic PAC Ca^{2+} response to CCK, in the presence or absence of 10 µM TRO40303 (T10)

Ca^{2+} changes in response to CCK (10 nM) show typical large, rapid rise followed by a plateau. Pre-treatment with T10 did not affect the initial rise in rise in but dampened the plateau phase. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) $\pm$ SEM, with respective number of recordings shown in the key.

6.3.2.2 *Mitochondrial and Ca^{2+} responses in the presence of 1 µM TRO40303*

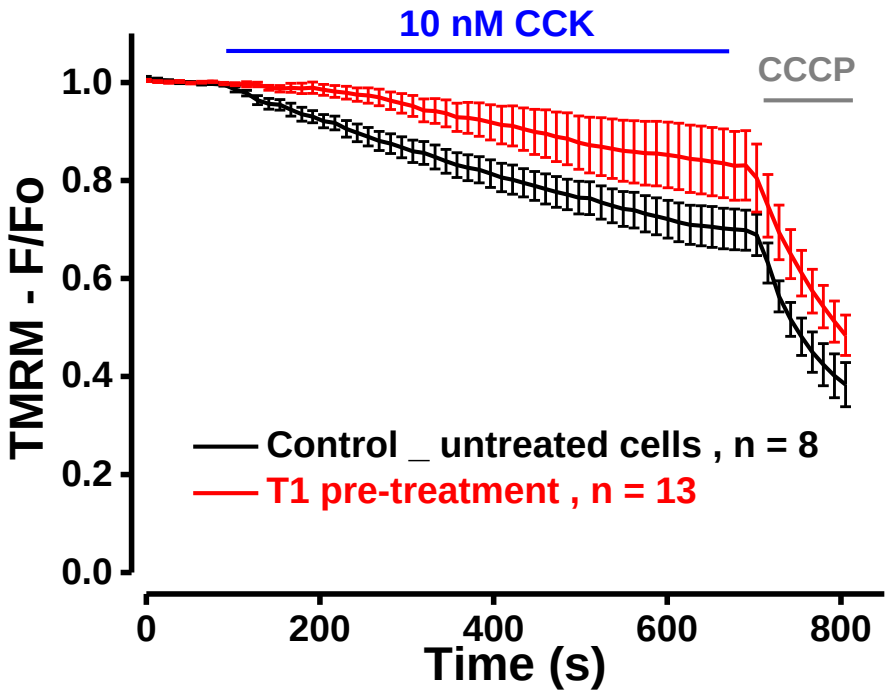


Figure 6.11- Pathologic PAC $\Delta\psi_m$ responses to CCK, in the presence or absence of 1 µM TRO40303 (T1)

$\Delta\psi_m$ changes in response to CCK (10 nM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T1 decreased the level of depolarisation partially. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

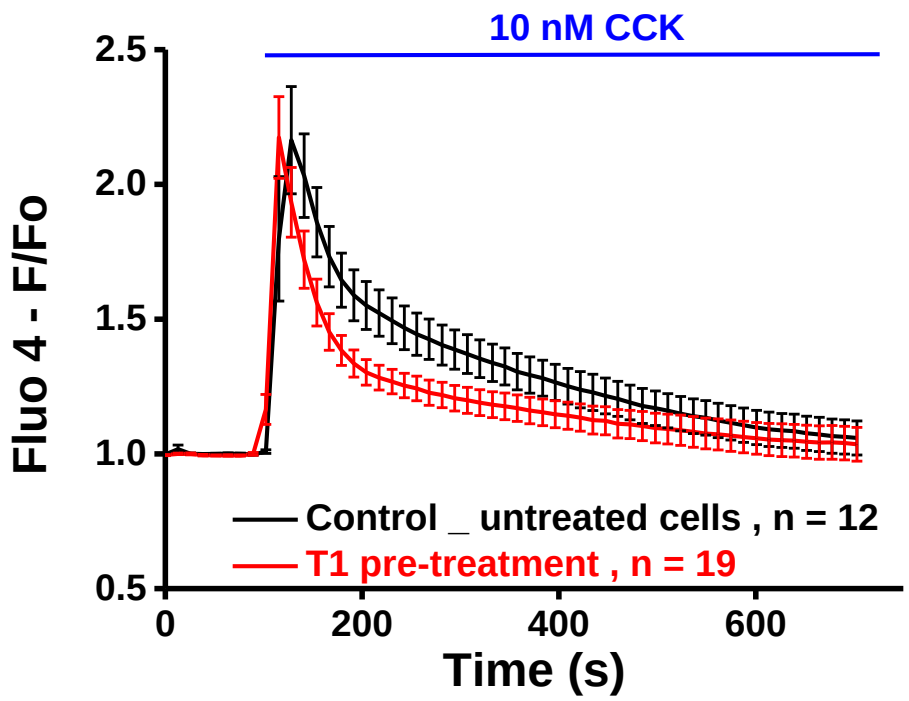


Figure 6.12 - Pathologic PAC Ca^{2+} responses CCK, in the presence or absence of 1 μM TRO40303 (T1)

Ca^{2+} changes in response to CCK (10 nM) show typical large, rapid rise followed by a plateau. Pre-treatment with T1 did not affect the initial rise in rise in but marginally reduced the plateau phase. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.3 Acinar cell responses to POAEE in the presence of TRO40303

6.3.3.1 Mitochondrial and Ca^{2+} responses in the presence of 10 µM TRO40303

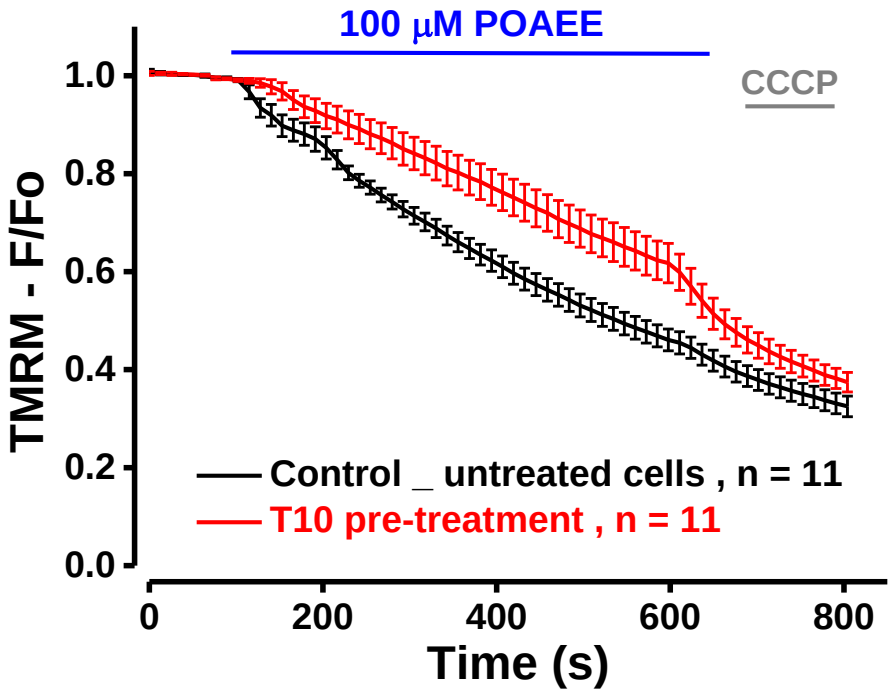


Figure 6.13 - Pathologic PAC $\Delta\psi_m$ responses to POAEE, in the presence or absence of 10 µM TRO40303 (T10)

$\Delta\psi_m$ changes in response to POAEE (100 µM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T10 partially decreased the level of depolarisation. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

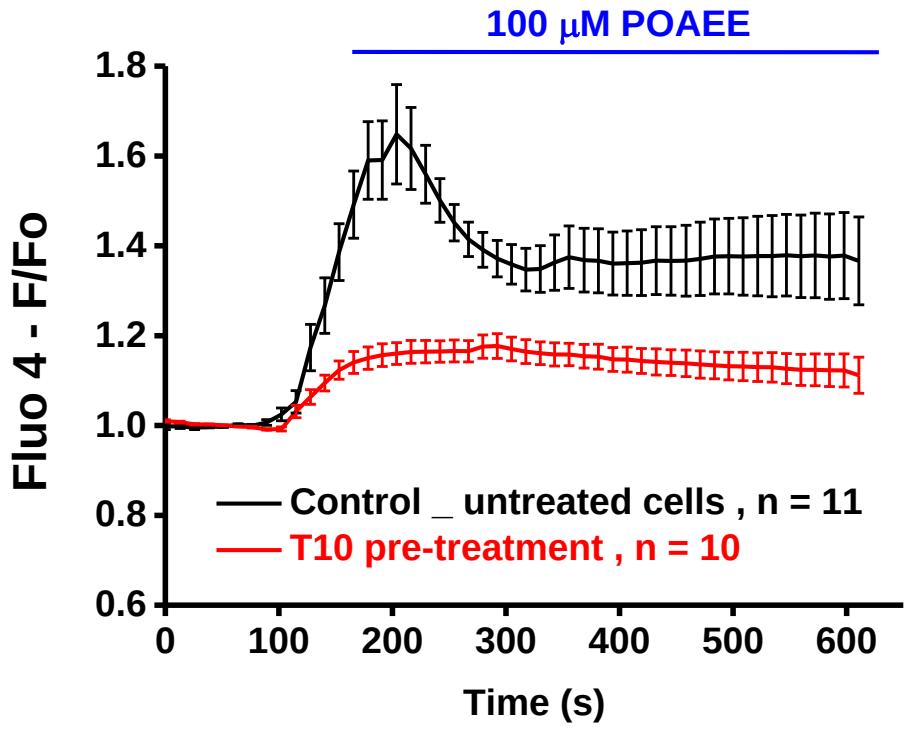


Figure 6.14 - Pathologic PAC Ca^{2+} responses to POAEE, in the presence or absence of 10 μM TRO4O303 (T10)

Ca^{2+} changes in response to POAEE (100 μM) show typical large, rapid rise followed by a plateau. Pre-treatment with T10 reduced the rise in cytosolic calcium. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.3.2 *Mitochondrial and Ca^{2+} responses in the presence of 1 µM TRO40303*

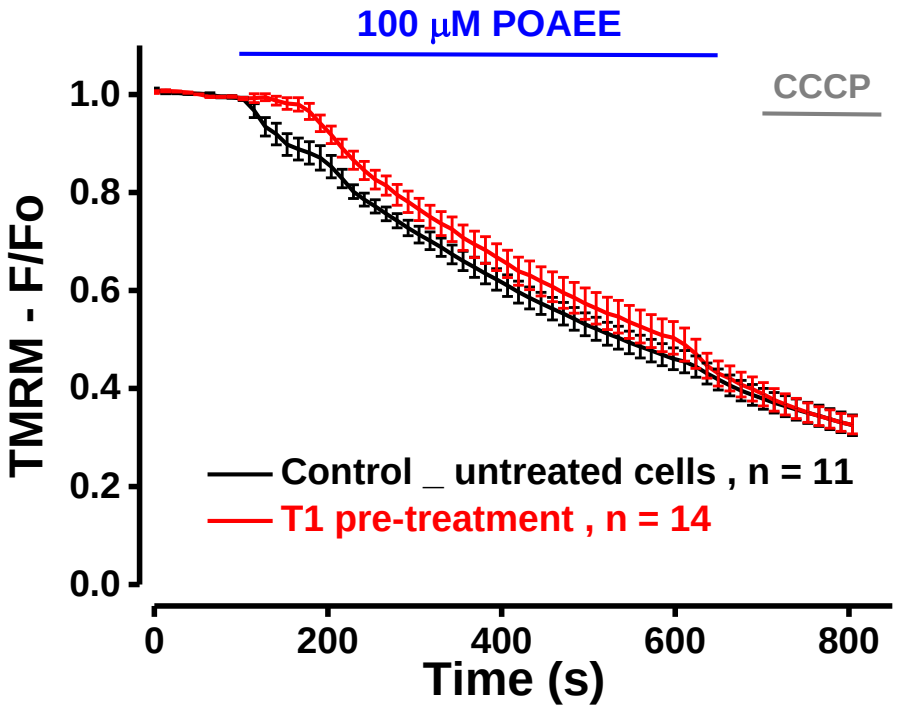


Figure 6.15 - Pathologic PAC $\Delta\psi_m$ responses to POAEE, in the presence or absence of 1 µM TRO40303 (T1)

$\Delta\psi_m$ changes in response to POAEE (100 µM) show significant depolarisation with complete depolarisation on application of protonophore CCCP, used as a positive control. Pre-treatment with T1 had no effect on the level of depolarisation. Mean changes in TMRM fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

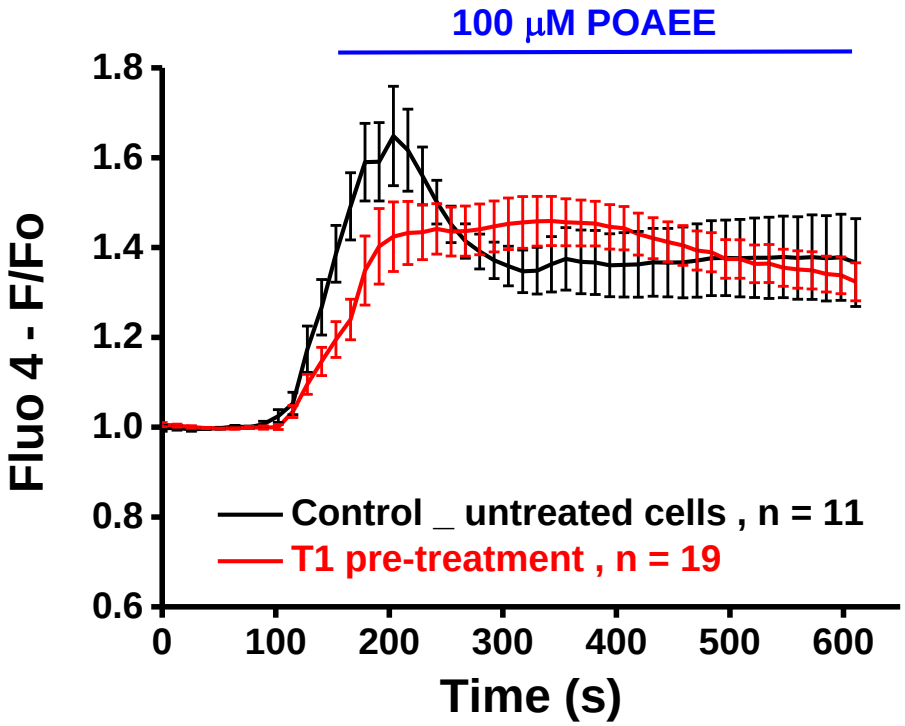


Figure 6.16 - Pathologic PAC Ca^{2+} responses to POAEE, in the presence or absence of 1 μM TRO40303 (T1)

Ca^{2+} changes in response to POAEE (100 μM) show typical large, rapid rise followed by a plateau. Pre-treatment with T1 did not have a significant effect on the rise in cytosolic calcium. Mean changes in Fluo-4 fluorescence, from at least three biological replicates, over baseline are shown (F/Fo) ± SEM, with respective number of recordings shown in the key.

6.3.4 Effects of TRO40303 on necrotic cell death pathway activation in response to POAEE in murine pancreatic acinar cells

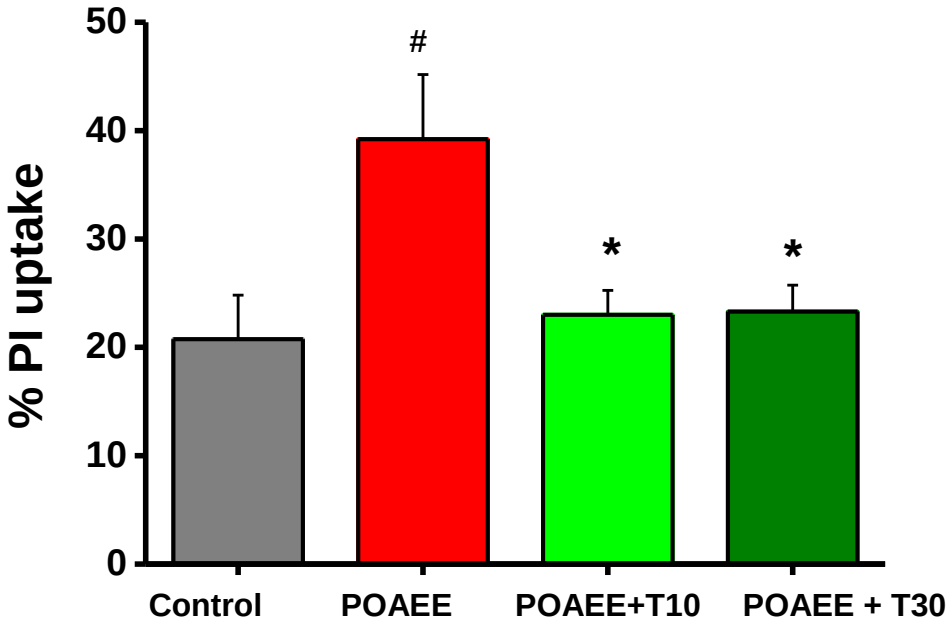


Figure 6.17 - PI uptake in response to POAEE, in the presence or absence of TRO40303

Co - administration of T10 and T30 with POAEE (100μM dissolved in 5 % ethanol) protected cells from early plasma membrane rupture (PI uptake) compared to untreated cells (n=2) *P<0.05, unpaired t- test.

6.3.5 Evaluation of binding of TRO40303 with CypD

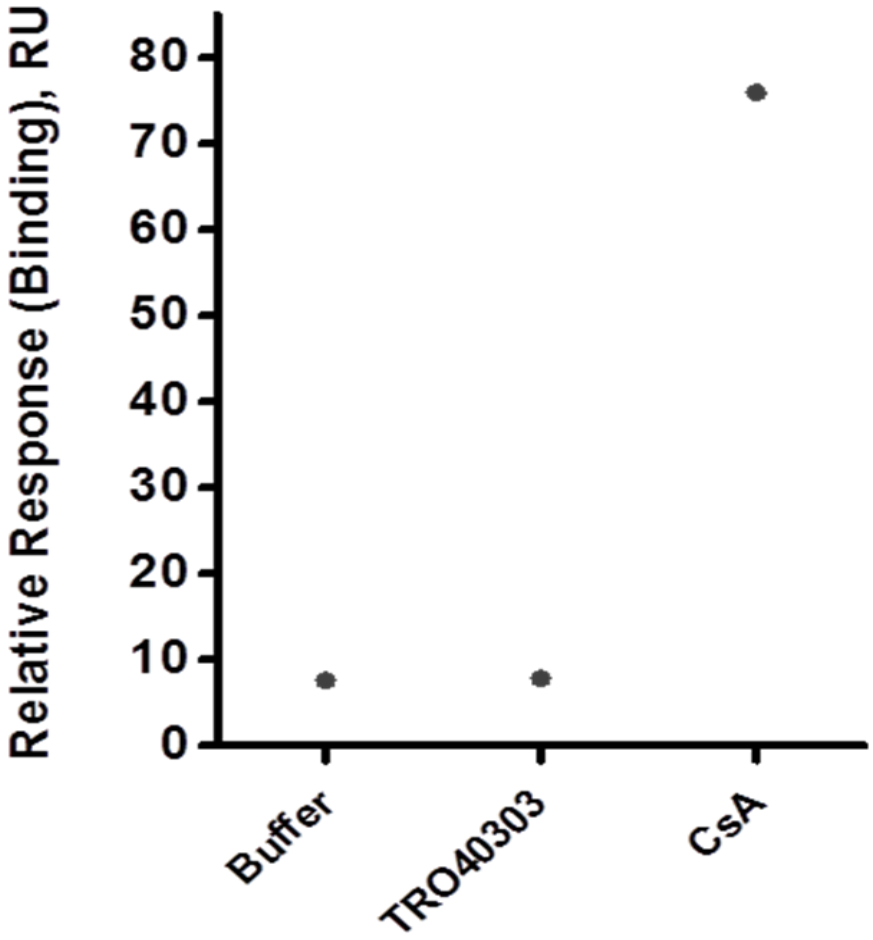


Figure 6.18 - SPR analysis of binding of TRO40303 to CypD

Surface plasmon resonance demonstrated no binding affinity of TRO40303 with CypD. Experiments were performed by the addition of 100µM TRO40303 over recombinant CypD immobilized on a CM5 sensor chip. TRO40303 resulted in the same response as buffer. CsA, which has a strong binding affinity for CypD, was used as a positive control. TRO40303 showed no binding with CypD.

6.3.6 Effect of TRO40303 treatment on severity of caerulein induced acute pancreatitis

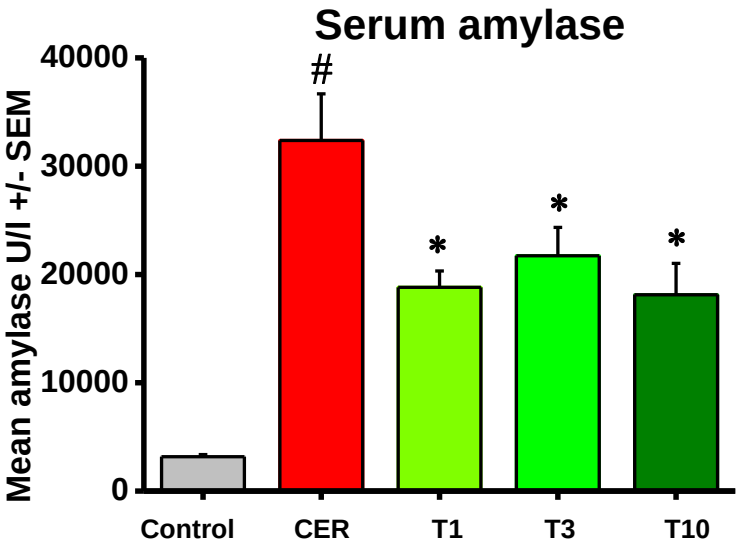


Figure 6.19 - Serum amylase measurements in CER-AP

Mean serum amylase is significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = 2.43 * 10^{-5}, T1 = 0.007, T3 = 0.026, T10 = 0.007.

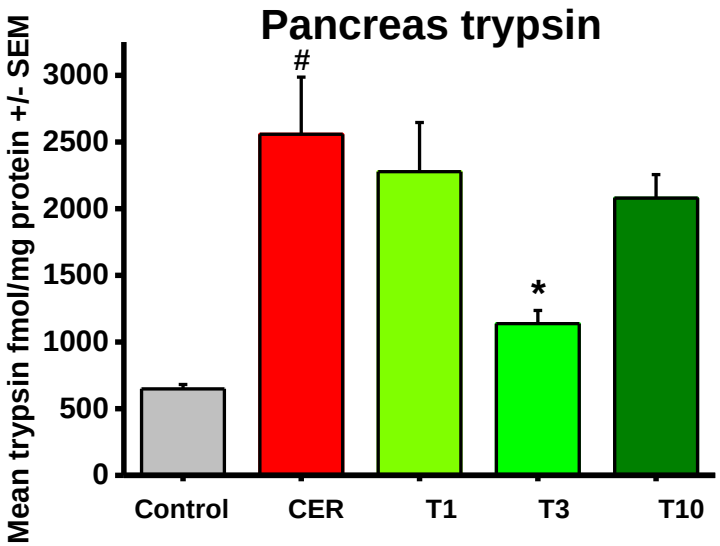


Figure 6.20 - Pancreatic trypsin measurements in CER-AP

Pancreatic tryspin is significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = 0.005, T1 = 0.32, T3 = 0.014, T10 = 0.17.

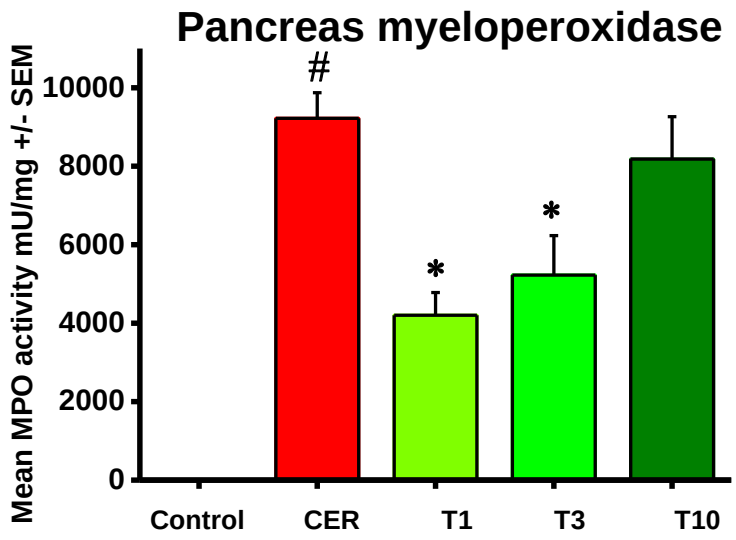


Figure 6.21 - Pancreatic myeloperoxidase measurements mice in CER-AP

Pancreatic myleoperoxidase activity as a measure of pancreatic neutrophil infiltration is significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = 1.58* 10^{-5}, T1 = 9.57* 10^{-5}, T3 = 0.0047, T10 = 0.22.

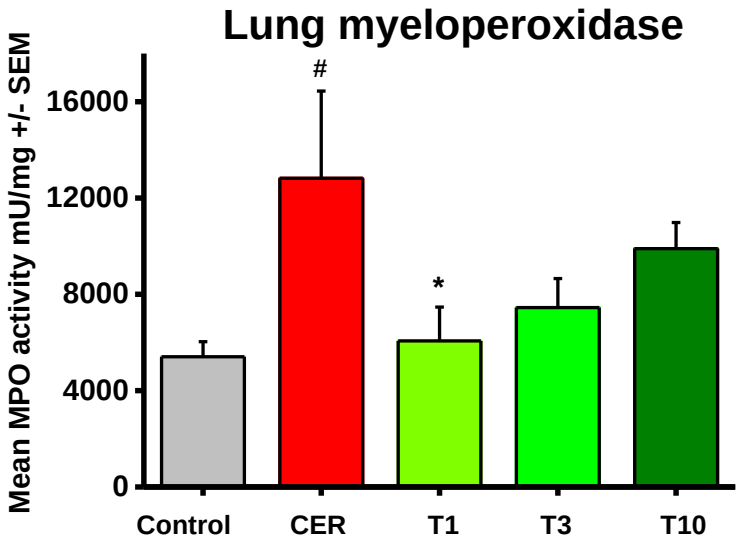


Figure 6.22 - Lung myeloperoxidase measurements in CER-AP

Lung myleoperoxidase activity as a measure of pancreatic neutrophil infiltration is significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = 0.048, T1 = 0.06, T3 = 0.10, T10 = 0.23.

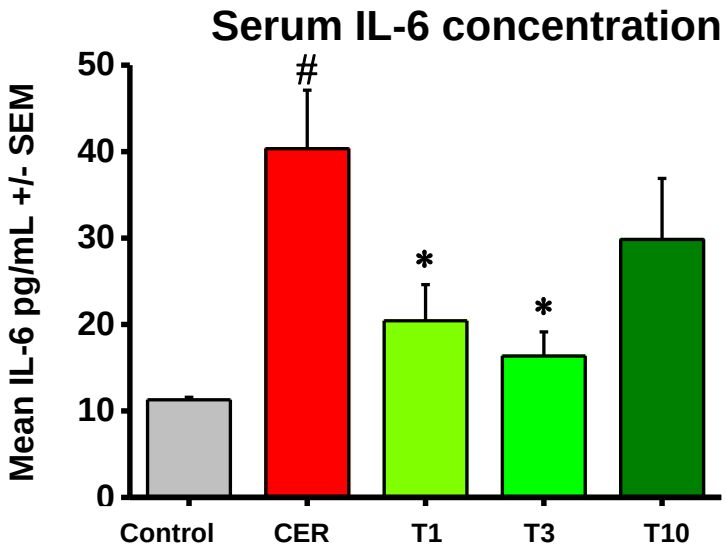


Figure 6.23 - Serum IL-6 measurements in CER-AP

IL-6 activity a pro-inflammatory cytokine is significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = 0.0026, T1 = 0.016, T3 = 0.006, T10 = 0.155.

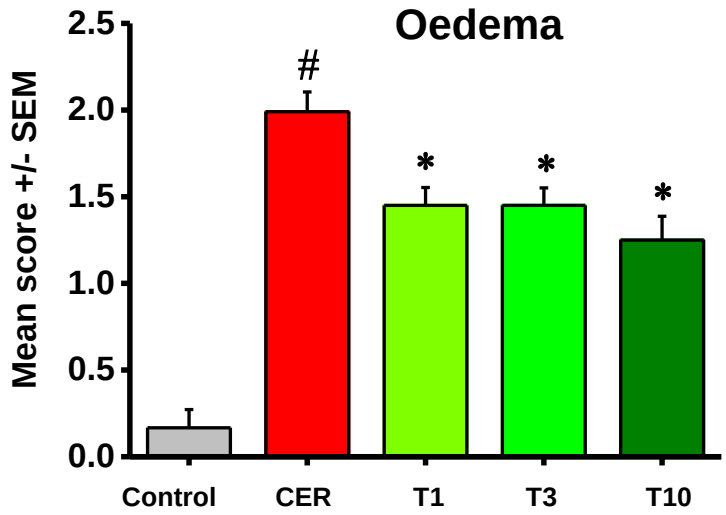


Figure 6.24 - Oedema scores in CER-AP

Oedema scores were significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = $1.01^{*}\ 10^{-8}$, T1 = 0.0018, T3 = 0.0017, T10 = 0.0011. All values are mean $\pm$ SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

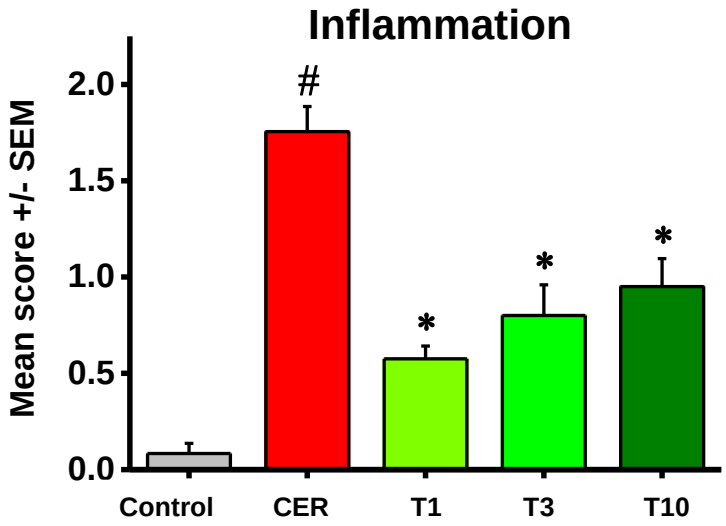


Figure 6.25 - Inflammation scores in CER-AP

Inflammation scores were significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = $3.83^{*}\ 10^{-8}$, T1 = $1.2^{*}\ 10^{-6}$, T3 = 0.00034, T10 = 0.001.All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

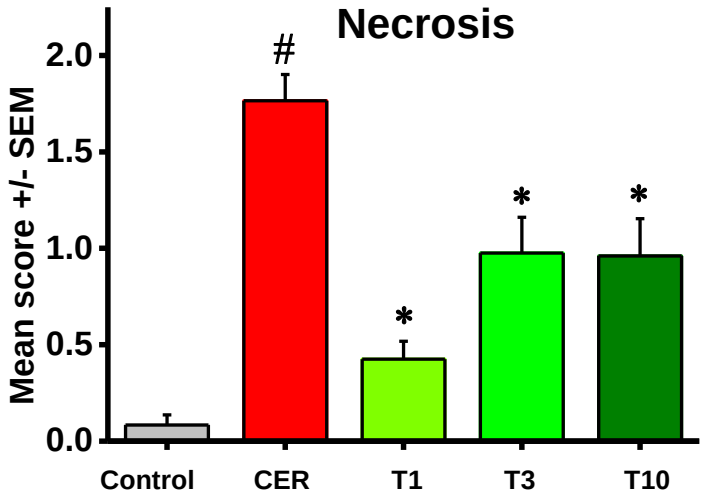


Figure 6.26 - Necrosis score scores in CER-AP

Necrosis scores were significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = $6.25^{*}\ 10^{-8}$, T1 = $6.05^{*}\ 10^{-7}$, T3 = 0.003, T10 = 0.0046. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

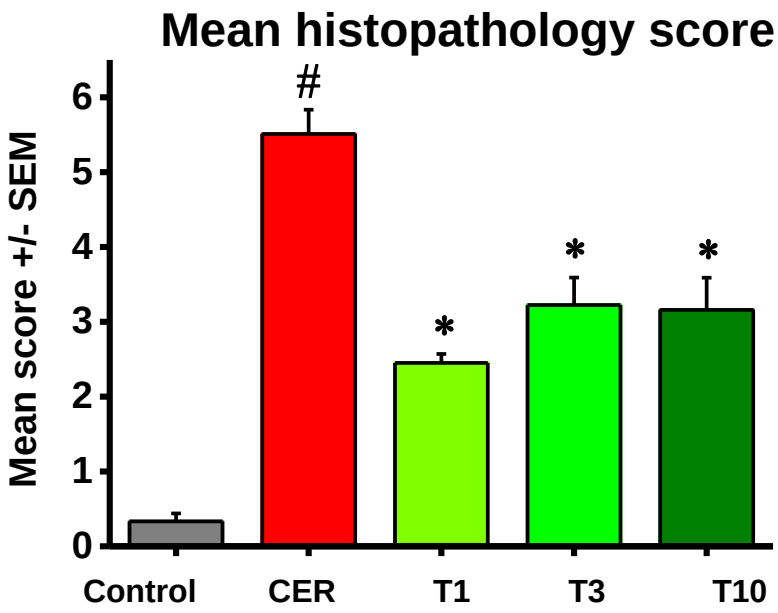


Figure 6.27 - Overall pancreatitis histopathology scores in CER-AP

Overall pancreatitis histopathology scores were significantly elevated in acute pancreatitis induced by caerulein in untreated mice and reduced in animals treated with TRO40303 (T1 = 1mg/kg, T3 = 3mg/kg, T10 = 10 mg/kg). P values for each group: CER = $6.39^{*}\ 10^{-9}$, T1 = $1.05^{*}\ 10^{-6}$, T3 = 0.00028, T10 = 0.0009.All values are mean $\pm$ SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

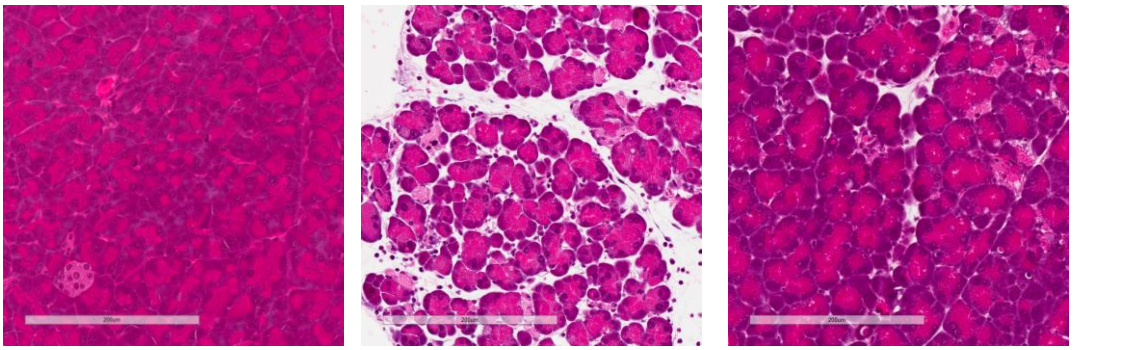


Figure 6.28 - Representative histopathology for CER-AP with TRO40303

Haematoxylin and Eosin stained X 200 magnification pancreatic sections showing significant histological features of acute pancreatitis characterized by ; oedema, neutrophil infiltration and necrosis produced by hyperstimulation model. Significant improvements in appearances were seen following TRO40303 treatment (3mg/kg) administered one hour after the induction of experimental acute pancreatitis.

6.3.7 Effect of TRO40303 treatment on severity of TLCS induced acute pancreatitis

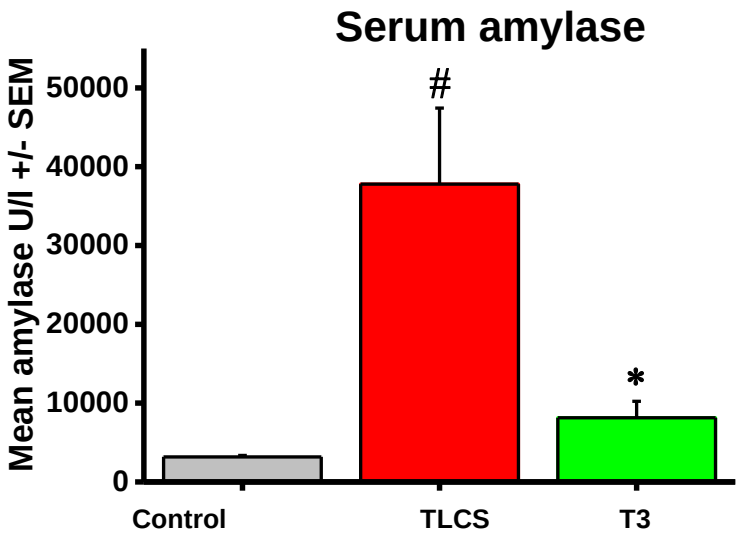


Figure 6.29 - Serum amylase measurements in TLCS-AP

Mean serum amylase is significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.0078, T3 = 0.013

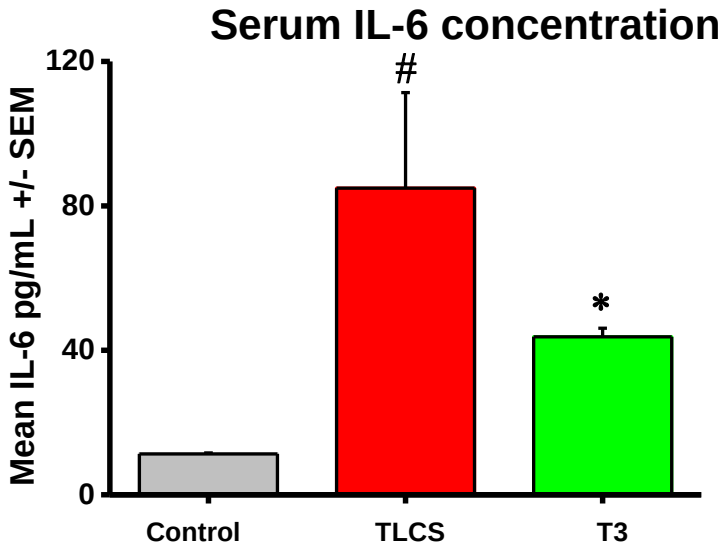


Figure 6.30 - Serum IL-6 measurements in TLCS-AP

Serum IL-6, a pro-inflammatory cytokine, is significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.012, T3 = 0.08.

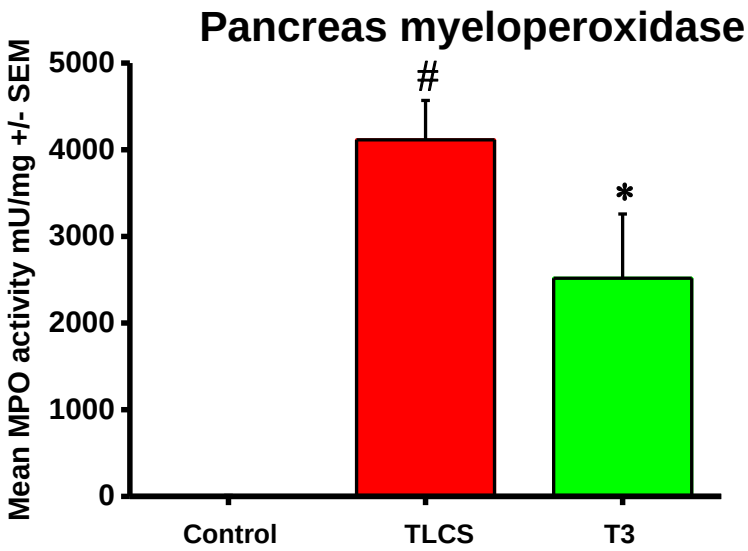


Figure 6.31 - Pancreas myeloperoxidase measurements TLCS-AP

Pancreatic myleoperoxidase activity, a measure of pancreatic neutrophil infiltration, is significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.00014, T3 = 0.048.

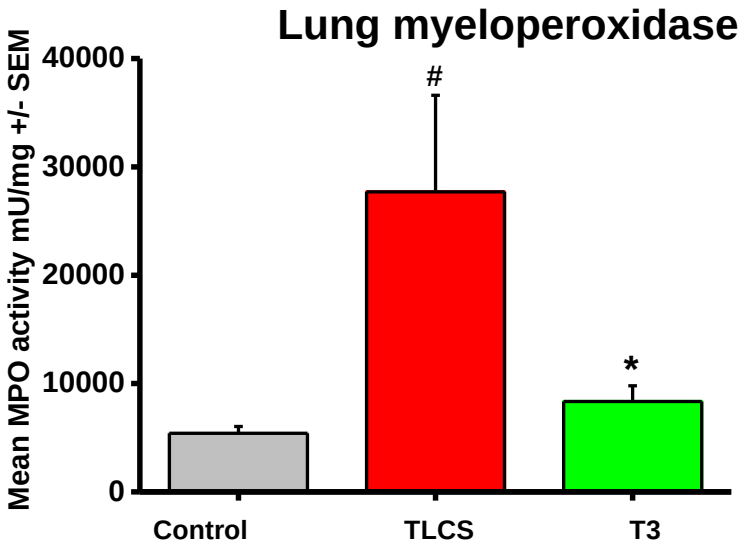


Figure 6.32 - Lung myeloperoxidase measurements in TLCS-AP

Lung myleoperoxidase activity, a measure of neutrophil infiltration, is significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.027, T3 = 0.041.

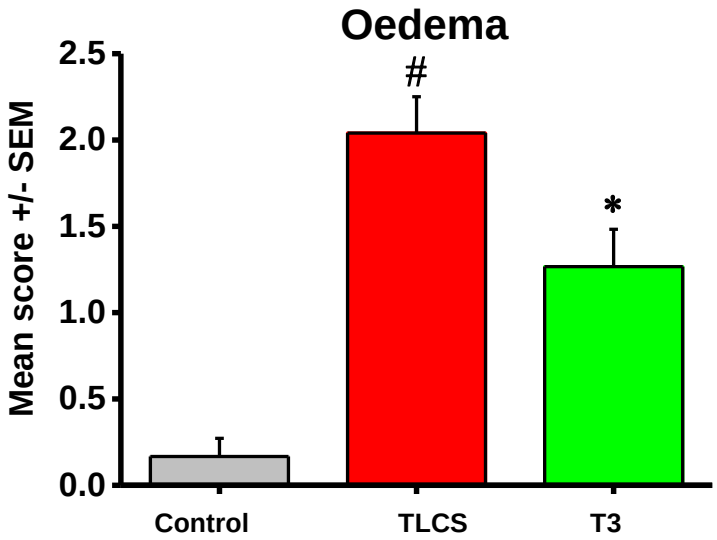


Figure 6.33 - Oedema scores in TLCS-AP

Oedema scores were significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.0001, T3 = 0.015. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

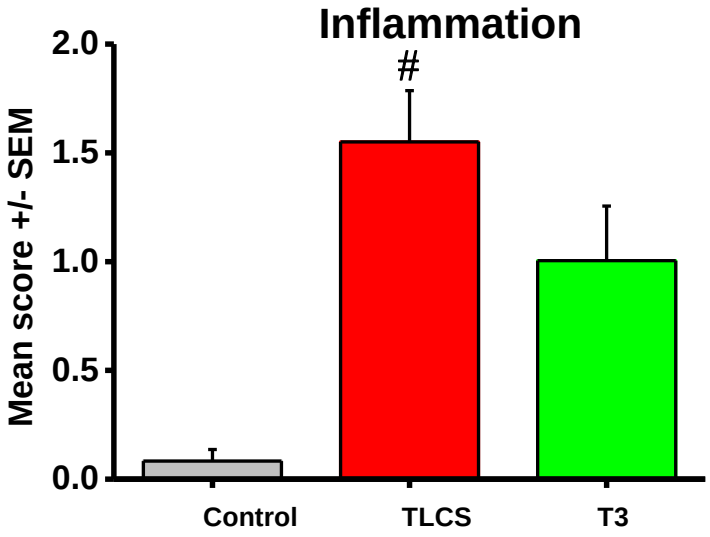


Figure 6.34 - Inflammation scores in TLCS-AP

Inflammation scores were significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.0014, T3 = 0.074. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

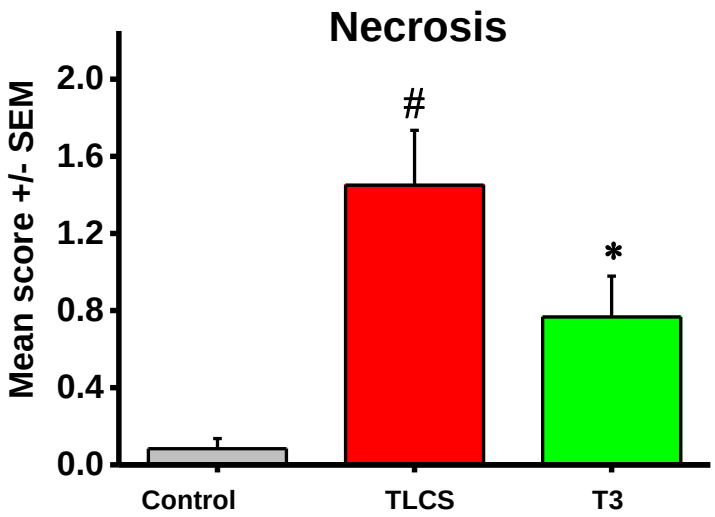


Figure 6.35 - Necrosis scores in TLCS-AP

Necrosis scores were significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.0039, T3 = 0.045. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

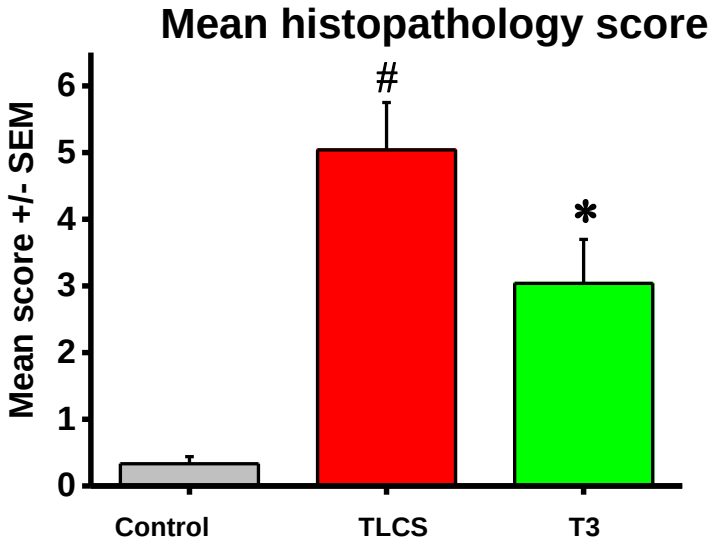
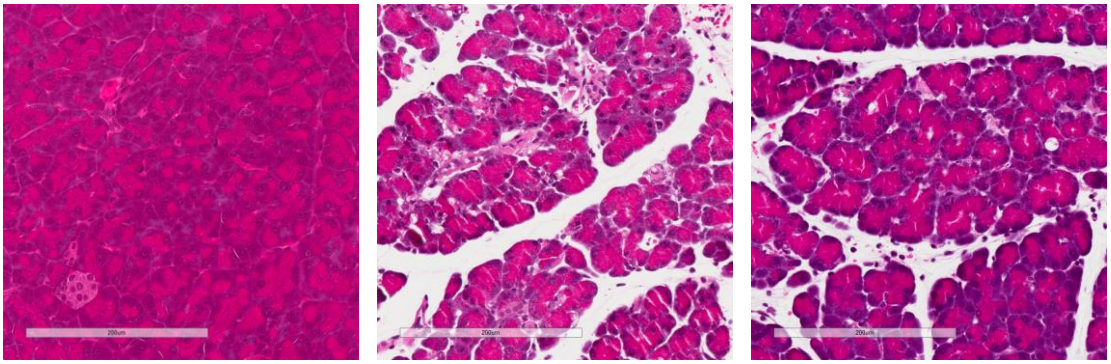


Figure 6.36 - Overall pancreatitis histopathology scores in TLCS-AP

Overall pancreatitis histopathology scores were significantly elevated in TLCS induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: TLCS = 0.0012, T3 = 0.035. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.



Control **TLCS-AP** **TLCS-AP + TRO40303, 3 mg/kg**

Figure 6.37 - Representative histopathology for TLCS-AP with TRO40303

Haematoxylin and Eosin stained X 200 magnification pancreatic sections showing significant histological features of acute pancreatitis characterized by ; oedema, neutrophil infiltration and necrosis produced by TLCS model. Significant improvements in appearances were seen following TRO40303 treatment (3mg/kg) administered one hour after the induction of experimental acute pancreatitis.

6.3.8 Effect of TRO40303 treatment on severity of alcoholic acute pancreatitis

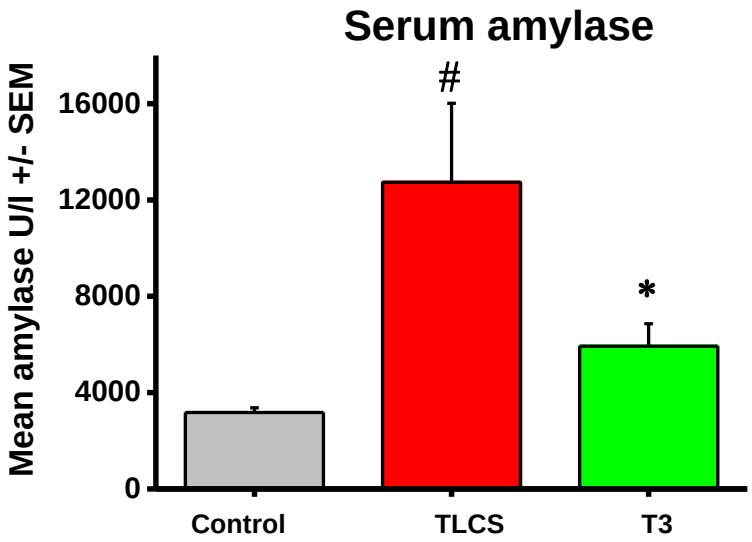


Figure 6.38 - Serum amylase measurements in FAEE-AP

Mean serum amylase is significantly elevated in FAEE acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.013, T3 = 0.043.

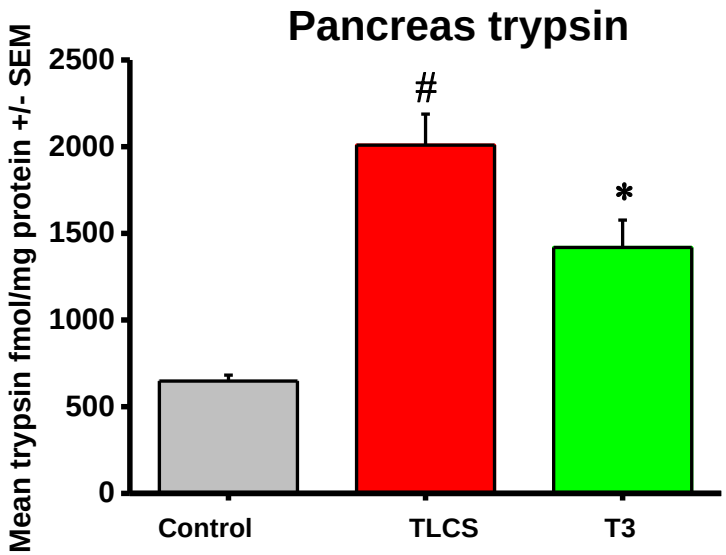


Figure 6.39 - Pancreatic trypsin measurements in FAEE-AP

Mean pancreatic trypsin is significantly elevated in FAEE acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.00025, T3 = 0.018.

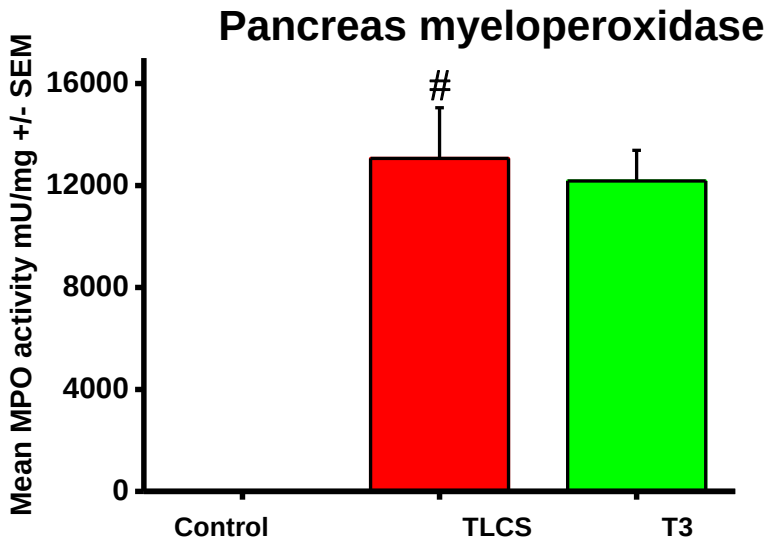


Figure 6.40 - Pancreatic myeloperoxidase measurements in FAEE-AP

Pancreatic myleoperoxidase activity as a measure of pancreatic neutrophil infiltration is significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = $1.32^{*}\ 10^{-5}$, T3 = 0.354.

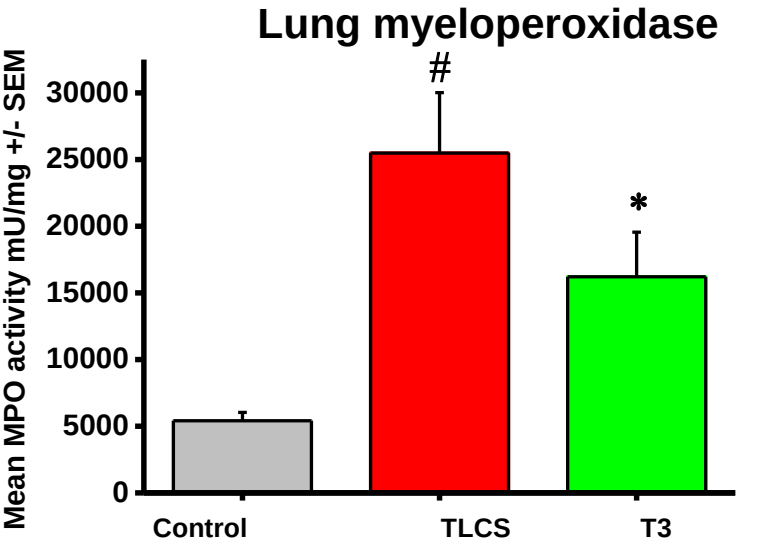


Figure 6.41 - Lung myeloperoxidase measurements in FAEE-AP

Lung myleoperoxidase activity as a measure of neutrophil infiltration is significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.0003, T3 = 0.0059.

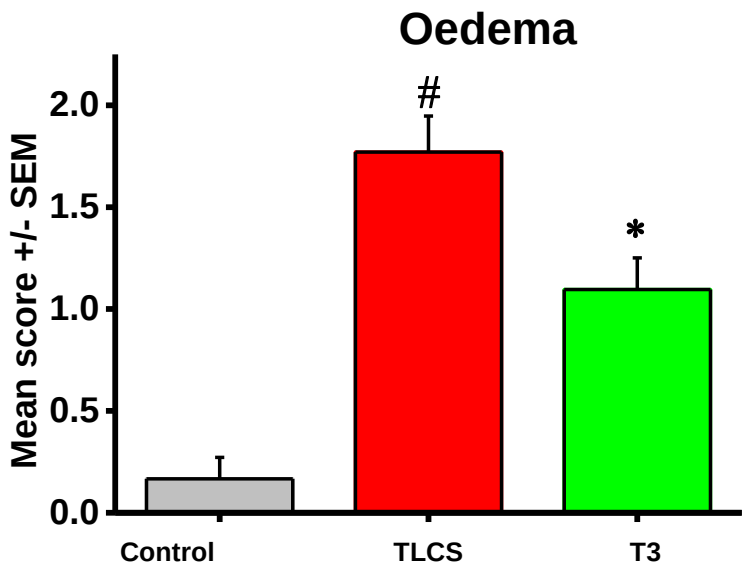


Figure 6.42 - Oedema scores in FAEE-AP

Oedema scores were significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in mice treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = $9.84*10^{-6}$, T3 = 0.0078. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

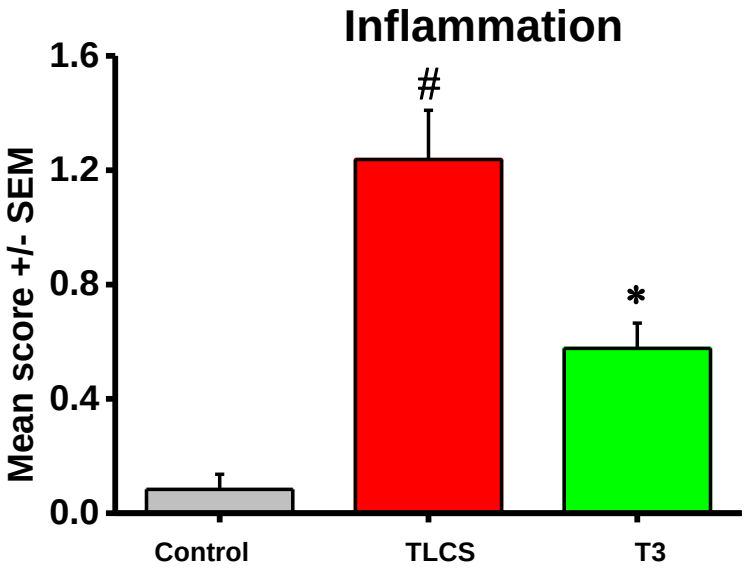


Figure 6.43 - Inflammation scores in FAEE-AP

Inflammation scores were significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in mice treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.00017, T3 = 0.0039. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

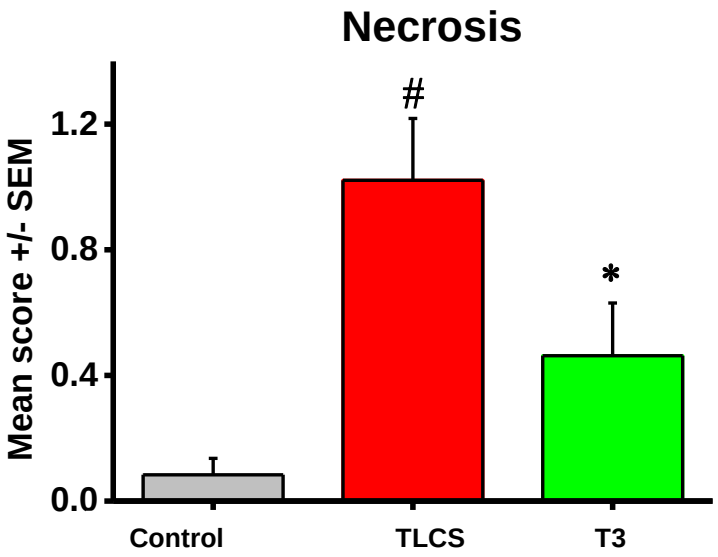


Figure 6.44 - Necrosis scores in FAEE-AP

Necrosis scores were significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.0013, T3 = 0.00269. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.

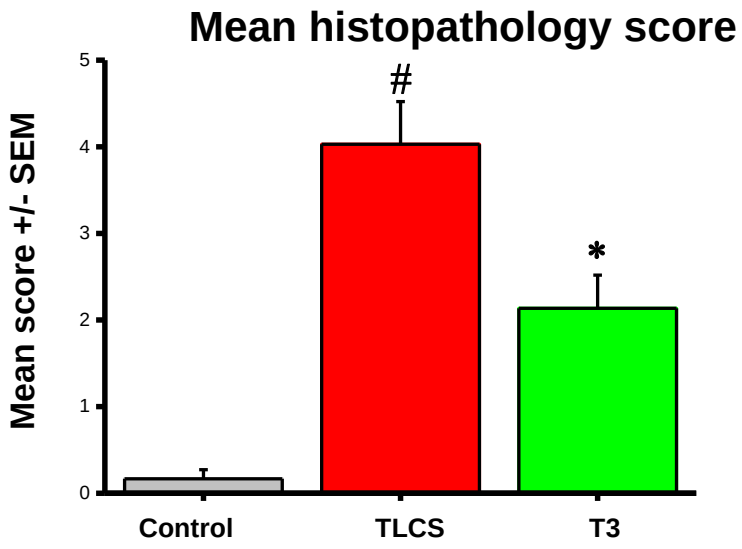
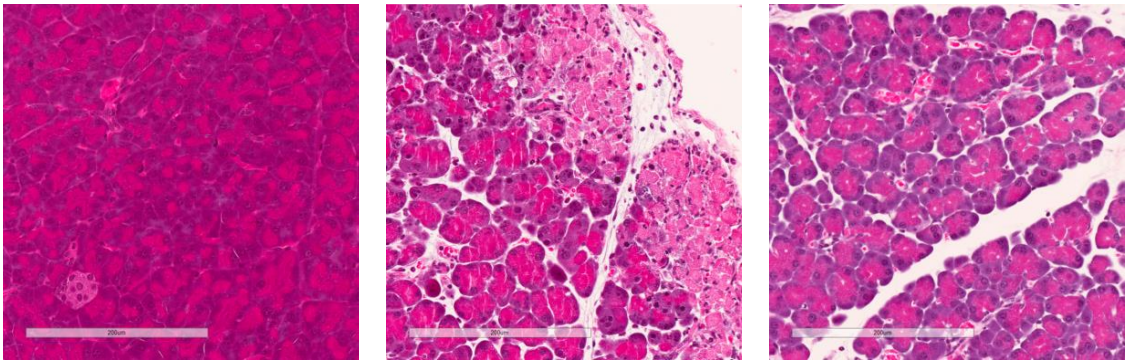


Figure 6.45 - Overall pancreatitis histopathology scores in-FAEE-AP

Overall pancreatitis histopathology scores were significantly elevated in FAEE induced acute pancreatitis in untreated mice and reduced in animals treated with TRO40303 (T3 = 3mg/kg). P values for each group: FAEE + Etoh = 0.001, T3 = 0.0059. All values are mean ± SEM from 10 fields scored per mouse with the average taken between two independent, blinded investigators.



Control **FAEE-AP** **FAEE-AP + TRO40303, 3 mg/kg**

Figure 6.46 - Representative histopathology for FAEE-AP with TRO40303

Haematoxylin and Eosin stained X 200 magnification pancreatic sections showing significant histological features of acute pancreatitis characterized by ; oedema, neutrophil infiltration and necrosis produced by TLCS model. Significant improvements in appearances were seen following TRO40303 treatment (3mg/kg) administered one hour after the induction of experimental acute pancreatitis.

6.3.9 TRO40303 levels in plasma and pancreas

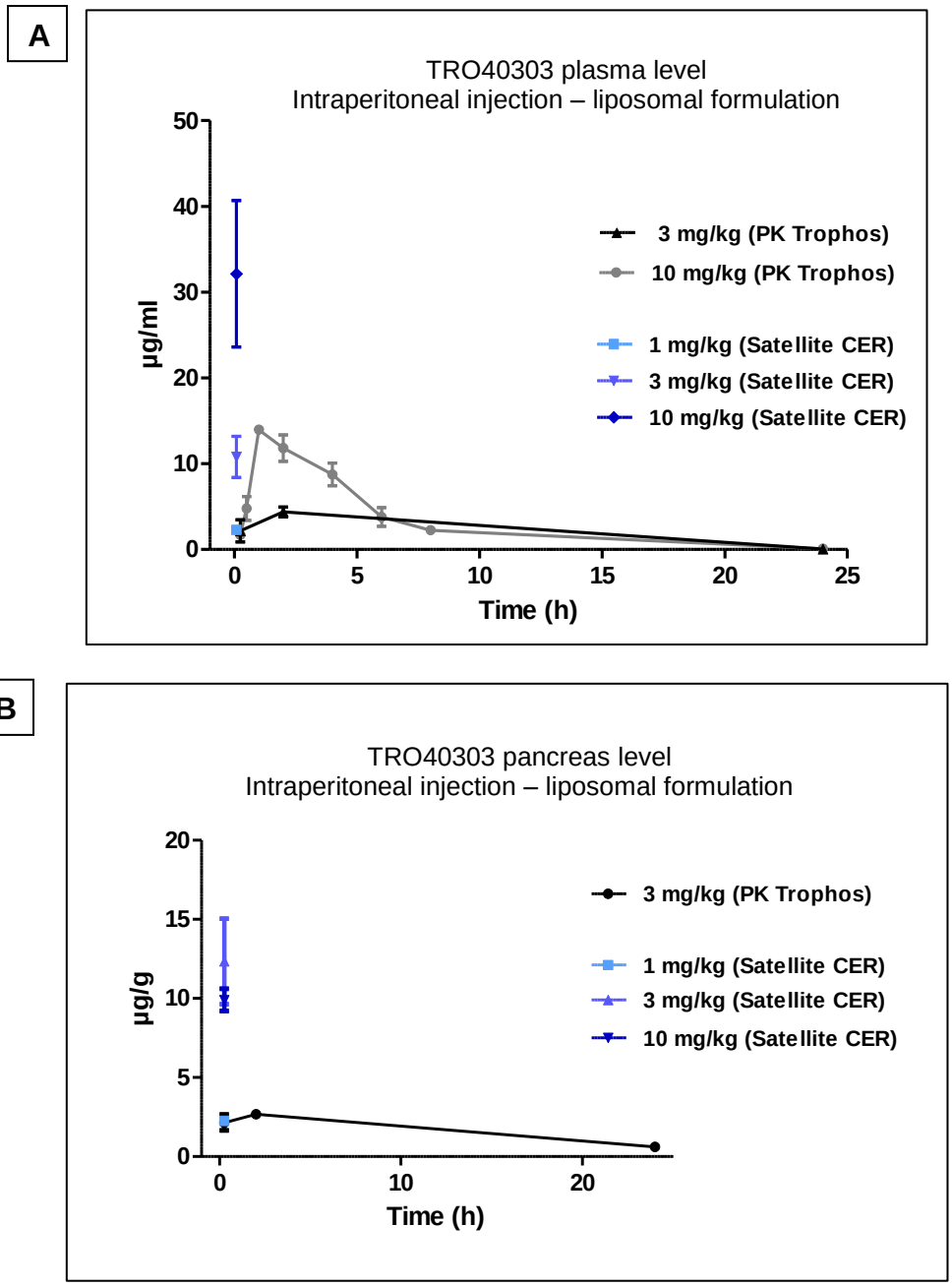


Figure 6.47 - Pharmacokinetics of TRO40303

(A) Plasma level of TRO40303 expressed in µg/ml during 24 hr after intraperitoneal administration to satellite mice (PK Trophos) of 3 and 10 mg/kg in liposomes compared to plasma level of TRO40303 15 min post administration in the caerulein model (satellite CER). (B) Pancreata level of TRO40303 expressed in µg/g tissue during 24 hr after administration to satellite mice (PK Trophos) of 3 mg/kg in liposomes compared to plasma level of TRO40303 15 min post administration in the caerulein model (satellite CER).

6.4 Discussion

Mounting evidence places mitochondrial injury centre stage in the pathogenesis of AP (Maleth, Rakonczay et al. 2013). MPTP inhibition is therefore an attractive strategy for developing a treatment for AP. Since CypD - a mitochondrial matrix protein - is considered to be the key regulator of MPTP formation (Giorgio, Bisetto et al. 2009, Giorgio, von Stockum et al. 2013, Mukherjee, Mareninova et al. 2015) CypD inhibitors have been evaluated and shown to reduce pancreatic injury *in vitro* and *in vivo* in response to pancreatic toxins (Shalbueva, Mareninova et al. 2013, Mukherjee, Mareninova et al. 2015). The experiments reported in this chapter demonstrate the protective effects of TRO40303 - a drug which inhibits MPTP formation by targeting mitochondrial translocator protein (TSPO) on the outer mitochondrial membrane (Schaller, Paradis et al. 2010) - on PAC injury developing in response to pancreatic toxins, which is a novel finding.

FAEEs as well as TLCS induce toxic sustained calcium overload in PACs which leads to mitochondrial dysfunction and necrosis (Criddle, Raraty et al. 2004, Criddle, Murphy et al. 2006). Although not targeting calcium overload per se, TRO40303 inhibits the critical downstream effects on the mitochondria, confers mitochondrial protection and reduces cellular injury (Schaller, Paradis et al. 2010, Le Lamer, Paradis et al. 2014, Schaller, Michaud et al. 2015). Pre-treatment with TRO40303 reduced mitochondrial dysfunction, cytosolic calcium overload and activation of necrotic cell death pathway in response to pancreatic toxins including TLCS, CCK and FAEEs in isolated murine PACs. The

protective effect of TRO40303 on TLCS induced necrosis in freshly isolated human PACs further supports the translational potential of this promising agent.

Therapeutic administration of TRO40303 intraperitoneally (3 mg/kg in liposomes) ameliorated the severity of CER-AP, TLCS-AP as well as FAEE-AP *in vivo*. The cumulative data on the effects of TRO40303 contrasts with previous preclinical trials of drugs for AP, the majority of which have been conducted in a single model only (most often CER-AP) (Pandol, Saluja et al. 2007) with treatment given prophylactically before disease induction. In the clinical setting, however, prophylactic administration of a drug is not possible in the majority of patients developing AP. The combination of ethanol with POA (Huang, Booth et al. 2014) as well as bile acid induced EAP (Laukkarinen, Van Acker et al. 2007) induce significant pancreatic damage with extensive acinar cell oedema, neutrophil infiltration and necrosis. The TLCS-AP model is considered to be representative of biliary acute pancreatitis in humans and has been extensively utilised in the past. The FAEE-AP model which has been reported recently is also considered to be clinically relevant. High levels of FAEEs accumulate in the pancreas following acute alcohol intoxication, in contrast to other organs commonly damaged by alcohol, (Laposata and Lange 1986) and substantial evidence now implicates FAEEs in PAC (Criddle, Raraty et al. 2004, Criddle, Murphy et al. 2006, Gerasimenko, Gryshchenko et al. 2013) as well as pancreatic ductal cell injury (Maleth, Balazs et al. 2015). Epidemiological studies have also shown a marked increase in the level of alcohol consumption in recent decades, which parallels a sustained rise in the incidence of AP (Yadav and Lowenfels 2013). The reduction in biochemical as

well as histological markers of severity in three different models of EAP on therapeutic administration therefore highlights the efficacy of TRO40303.

The mitochondrion is the powerhouse of the cell. There is evidence indicating that sepsis-induced multiple organ failure depends on the development of mitochondrial dysfunction and consequent cellular energetic failure (Duran-Bedolla, Montes de Oca-Sandoval et al. 2014). Multiorgan failure is also the leading cause of death in patients with severe AP (Petrov, Shanbhag et al. 2010). TRO40303 is an MPTP modulator that has been shown to be protective in multiple cellular and animal models characterised by stress-induced mitochondrial permeabilization,(Schaller, Paradis et al. 2010, Le Lamer, Paradis et al. 2014), (de Tassigny, Assaly et al. 2013, Richter, Gao et al. 2014) although whether TSPO binding is the primary mechanism of action remains to be confirmed (Sileikyte and Forte 2016). Pharmacokinetic profiles of TRO40303 have shown accumulation at substantial levels within the lungs, liver, heart and kidneys, (Schaller, Paradis et al. 2010) all organs contributing to organ failure in AP. Levels were higher in EAP than in naïve animals, possibly due to reduction in the speed of metabolism and/or excretion of the drug, a consideration in the design of any future human trials. Nevertheless the accumulation in vital organs suggests that TRO40303 might also usefully prevent or lessen systemic organ dysfunction in AP through direct effects on these organs, suggesting promise in this approach.

These findings strengthen an established rationale for targeting the MPTP to treat a range of acute and chronic conditions in which necrotic cell death is a critical and/or prominent feature (Bernardi, Rasola et al. 2015,

Sileikyte and Forte 2016). CsA is the only licensed drug used experimentally to inhibit formation of the MPTP, the mechanism of action being inhibition of CypD, the most widely recognised regulator of the MPTP. Clinical trials testing the efficacy of CsA in this regard have been undertaken, with mixed results: a pilot study found reduction in the size and severity of myocardial infarction associated with CsA treatment prior to percutaneous coronary intervention (Piot, Croisille et al. 2008), not replicated in a larger study (Cung, Morel et al. 2015). The immunosuppressive actions of CsA essential to its primary therapeutic use in controlling autoimmunity and allograft rejection, however, preclude widespread use in conditions where the MPTP is a target. For example AP predisposes to local and systemic infection, which contributes significantly to mortality (Petrov, Shanbhag et al. 2010, Afghani, Pandol et al. 2015); the powerful immunosuppressive actions of CsA are likely to impact negatively on infective outcomes in AP. There are continuing major efforts to develop new, specific inhibitors of CypD although none is ready for first in man study. TRO40303, which does not have the immunosuppressive actions of CsA but which has been shown experimentally to have a significant impact on a number of forms of organ injury, has also been tested in a phase II trial of cardiac preservation following acute myocardial infarction, but was not found to have significant protective effects (Atar, Arheden et al. 2015). While the mechanism of action of TRO40303 remains to be confirmed (Sileikyte and Forte 2016), the experimental data shows that TRO40303 did not bind to CypD. Nevertheless since TRO40303 may have both pancreatic as well as systemic protective effects in AP and the therapeutic window is potentially longer than in myocardial infarction, there is perhaps a higher chance of efficacy in AP than in

myocardial infarction. While phenotypic screens have identified new MPTP inhibitors and a number of powerful compounds have been identified, all present challenges: cinnamic anilides were found to be no more effective than CsA in experimental myocardial infarction, isoxasoles are unstable *in vivo* and benzamides impair ATP generation (Sileikyte and Forte 2016). The small number of agents in development for the treatment of AP and the extensive work already undertaken to develop TRO40303 as a drug support the case to test TRO40303 in human AP. The previously conducted animal toxicology studies for TRO40303 have been based on the assumption that the drug would be administered as a single dose for its clinical application in cardiac ischaemic-reperfusion injury prior to performing percutaneous coronary intervention in patients with myocardial infarction. The pre-clinical toxicity profile of TRO40303 would therefore have to be re-evaluated if it is to be used for the treatment of human AP as it would require repeated doses.

Chapter 7 – Integrated Discussion

There is an unmet clinical need for developing a treatment for AP as there is no licenced, targeted pharmacotherapy for this potentially life threating disease; the burden for which is increasing worldwide (Yadav and Lowenfels 2013). Positive findings from pre-clinical studies have not translated in human clinical trials with numerous negative RCTs (Pezzilli 2009). The main problems with translational drug discovery in AP have been; lack of clear consensus and elucidation of key pathological mechanisms implicated in the pathophysiology of disease and difficulty in designing clinical studies that can provide reliable, statistically significant answers. The PAC has long been established as the initial site of damage in AP (Pandol, Saluja et al. 2007), however the review of literature undertaken as part of this study reveals that this has not been the primary focus of strategies adopted for inhibition of EAP. Furthermore, the design of pre-clinical studies in EAP does not reflect the clinical picture in human AP, there is significant variability in the toxicity of models of EAP as well as the quality of reported studies. The poor translational power of animal models in stroke led to the establishment of the Stroke Therapy Academic Industry Roundtable, which issued recommendations to improve the quality of preclinical stroke research (Vaquero, Molero et al. 1999) and this initiative had a positive impact on the overall translational research strategy in ischaemic stroke. Perhaps a similar approach needs to be adopted by the pancreatic community. There is potentially a case for exploring and developing more clinically relevant experimental models of AP in higher mammals or non-human primates which would need to be closely monitored, regulated and restricted to highly specialised research facilities.

This study builds on the present knowledge regarding mitochondrial function in AP and demonstrates that MPTP inhibition prevents mitochondrial dysfunction in PACs and ameliorates the severity of EAP. Findings in the current study show that both genetic deletion of CypD and pharmacological inhibition of MPTP using a variety of agents (CsA, Deb025, AP-1A02 and TRO40303) preserve mitochondrial function and reduce necrotic cell death, the aftermath of mitochondrial dysfunction. These results are in keeping with literature and support that MPTP opening in PACs is the key determinant of loss of MMP in response to pancreatic toxins, as well as subsequent impairment of mitochondrial metabolism with a decrease of ATP for energy dependant cellular functions (Mukherjee, Mareninova et al. 2015). Data from normal human PACs also confirms the potential of experiments on isolated acinar cells obtained from normal human pancreatic tissue to discover key mechanisms and concordance with *in vitro* murine findings.

Although there has been considerable controversy with regards to the exact composition of MPTP, CypD has been the only protein that has consistently been considered to be the regulator of MPTP formation (Baines, Kaiser et al. 2005, Giorgio, Bisetto et al. 2009) and has indeed been a key component of this study. Using the target-based approach for drug discovery; a novel, small molecule CypD inhibitor (AP-1A02) has been identified which prevents mitochondrial dysfunction and significantly reduces bile acid induced necrotic cell death not only in murine but also in human PACs. This compound can form the basis of a lead optimization chemistry programme to increase potency of the chemical series. The partnership between the University of Liverpool and Cypralis - a life sciences company focused on PPIase modulators - to identify

and develop novel CypD inhibitors is an encouraging development in the field. Given the high sequence homology of cyclophilin family members and biological significance of the other cyclophilins in humans, it would be prudent to have a counter-screening programme with other cyclophilins, especially CypA and CypB to identify inhibitors that are selective for CypD. Such an approach would reduce the potential off-target effects of non-selective cyclophilin inhibitors. The biological assays using confocal microscopy to evaluate $\Delta\psi_m$ and activation of necrotic cell death pathway as part of this study, although extremely reliable, are time consuming. Other strategies can be adopted for high throughput screening of CypD inhibitors in the first instance and confocal microscopy can then be used at a later stage to evaluate and confirm the biological effects of MPTP inhibition on PACs. The mitochondrial calcium retention capacity (CRC) assay is one such method of evaluating MPTP opening (Fontaine, Eriksson et al. 1998) and further modifications of this technique (Yamaguchi, Andreyev et al. 2007) to isolate mitochondria and preserve function after freeze-thaw using the cryopreservative agent trehalose have been described which allow screening for novel MPTP inhibitors in a rapid and efficient manner. The use of rat AR42J cell line (Christophe 1994), the most commonly used cell line to study the exocrine pancreas, as opposed to freshly isolated murine PACs, for initial biological screening of MPTP inhibitors can also increase the throughput of identifying novel CypD inhibitors. The JC-10 $\Delta\psi_m$ assay (Kysenius, Brunello et al. 2014) can be performed in the format of a 96 microplate reader and provides an alternative, robust and efficient method for monitoring changes in $\Delta\psi_m$. JC-10 is a lipophilic, cationic dye which

concentrates in the mitochondrial matrix where it forms red fluorescent aggregates. However as the mitochondrial permeability increases, JC-10 diffuses out of mitochondria, changes to monomeric form and stains cells in green fluorescence (Sakamuru, Attene-Ramos et al. 2016). The lactate dehydrogenase assay (LDH) for evaluating cell death can also be performed on the 96 well plate reader and is therefore an efficient and reliable means of evaluating cell death in PACs. LDH catalyses the interconversion of pyruvate to lactate and NADH to NAD+ (Madern 2002). Elevated levels of LDH are indicative of tissue injury and breakdown. LDH can be measured using colorimetric assays supplied by Promega (Orabi, Muili et al. 2013).

This study provides a comprehensive evaluation of TRO40303, a drug quite far in clinical development, examining its efficacy in preventing toxin induced pancreatic injury *in vitro* and *in vivo*. The protective effects of TRO40303 in isolated murine PAC experiments were observed to be mirrored in three different models of EAP and also in human PACs. Although the phenomenon of MPTP inhibition by TRO40303 has been observed, the exact mechanism by which the drug modulates MPTP remains unclear. We have shown that TRO40303 does not bind to CypD. The proposed mechanism of action of TRO40303 that it inhibits MPTP formation as it binds with the TSPO located on the OMM has also been challenged. There is evidence from conditional knockout mice in which the TSPO gene has been eliminated in the liver and heart (Sileikyte, Blachly-Dyson et al. 2014) that the role of TSPO in the regulation of the MPTP in hepatocytes and cardiomyocytes is dispensable. There are however data indicating that 'TSPO ligands' bind to and inhibit the mitochondrial F_OF_1-ATP synthase (Stelzer, Frazee et al. 2010) which forms the

MPTP. It would therefore appear logical to utilise structural biological assays such as NMR to evaluate the binding between TRO40303 and OSCP binding site of the F_OF_1-ATP synthase as well as other components of the F_OF_1-ATP synthase to see whether TRO40303 directly inhibits the formation of MPTP.

Binding of TRO40303 with TSPO may indirectly modulate MPTP opening as TSPO could act on well-known activators of the pore such as reactive oxygen species, calcium and/or other proteins that have been involved in MPTP opening (Morin, Musman et al. 2016). TRO40303 could favour the VDAC - hexokinase interaction thereby facilitating a metabolic switch from oxidative phosphorylation to glycolysis and maintain ATP levels within the cell (Schaller, Paradis et al. 2010). Cardioprotection from TSPO ligands such as 4'-chlorodiazepam is associated with inhibition of mitochondrial accumulation of cholesterol at reperfusion (Paradis, Leoni et al. 2013). Rapid accumulation of cholesterol within the mitochondrial matrix, in the presence of reactive oxygen species can lead to the formation of a complex mixture of oxysterols which can induce MPTP opening. The effects of TRO40303 on VDAC - hexokinase interaction and mitochondrial accumulation of cholesterol within the PAC can be evaluated in future experiments to investigate the mechanism by which TRO40303 prevents MPTP inhibition.

Loss of $\Delta\psi_m$ leads to depletion of intracellular ATP levels and subsequent necrosis. Having demonstrated the preservation of mitochondrial function in response to pancreatic toxins, the effects of TRO40303 in maintaining ATP levels in PACs can be evaluated using a luciferin/luciferase luminescence-based assay. In order to examine the effects of the drug on mitochondrial calcium and intracellular levels of reactive oxygen species, the

dyes Rhod-2 and 2',7' – dichlorofluorescin diacetate (DCFDA) can be used and confocal microscopy experiments undertaken using the protocols described for $\Delta\psi_m$ and cytosolic calcium. NF-κB activation is thought to be an early and critical component of the inflammatory response during acute pancreatitis (Rakonczay, Hegyi et al. 2008). Traditional methods for examining NF-κB activity *in vitro* including protein determination of NF-κB pathway markers (phosphorylated IκB; p65 nuclear translocation; IKK upregulation), electromobility shift assay (EMSA) and immunohistochemistry for phosphorylated p65 (Algul, Tando et al. 2002) can also be used to study the effects of TRO40303 on the NF-κB pathway.

The two most important determinants of outcome in patients with AP are pancreatic necrosis and organ failure. TRO40303 has been shown to reduce activation of necrotic cell death pathway *in vitro* and ameliorate the severity of EAP; however its impact on organ failure can be investigated in further detail. There is increasing evidence that the intestine has a central role, both as culprit and victim, in severe AP (Flint and Windsor 2003) contributing to multi organ dysfunction syndrome (MODS). Pancreatic inflammation causes reflex splanchnic vasoconstriction which leads to ischaemia / reperfusion injury. This cascade of events results in gut mucosal injury, intestinal barrier failure, endotoxemia, and bacterial translocation which contribute to the SIRS response in AP. Bacterial translocation increases the risk of infected pancreatic necrosis (Windsor, Fearon et al. 1993) in cases of sterile pancreatic necrosis. There is also evidence to suggest that there is early organ-selective mitochondrial dysfunction in the lung and jejunum followed by the heart, liver and kidney (Mittal, Hickey et al. 2011) in EAP. Since TRO40303 is known to

prevent ischaemia/reperfusion injury in the heart, future experiments could evaluate the effect of therapeutic administration of the drug on mitochondrial dysfunction in different organs using mitochondrial spirometry. The *ex vivo* working heart model (Vidavalur, Swarnakar et al. 2008) and intra-vital microscopy for measuring microcirculation can also be used to study the effects of TRO40303 on cardiovascular system.

All in all the findings reported in this thesis confirm the role of MPTP formation in the pathogenesis of AP and pave the way for further development of TRO40303 and novel, small molecular CypD inhibitors, as potential drug candidates for the treatment of patients with acute pancreatitis.

Publications arising from this work

I. TRO40303 Ameliorates Alcohol-Induced Pancreatitis Through Reduction of Fatty Acid Ethyl Ester-Induced Mitochondrial Injury and Necrotic Cell Death.

Javed MA, Wen L, Awais M, Latawiec D, Huang W, Chvanov M, Schaller S, Bordet T, Michaud M, Pruss R, Tepikin A, Criddle D, Sutton R.

Pancreas. 2018 Jan;47(1):18-24.

II. Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP.

Mukherjee R, Mareninova OA, Odinokova IV, Huang W, Murphy J, Chvanov M, Javed MA, Wen L, Booth DM, Cane MC, Awais M, Gavillet B, Pruss RM, Schaller S, Molkentin JD, Tepikin AV, Petersen OH, Pandol SJ, Gukovsky I, Criddle DN, Gukovskaya AS, Sutton R.

Gut. 2016 Aug;65(8):1333-46

III. Small Molecule Inhibitors of Cyclophilin D To Protect Mitochondrial Function as a Potential Treatment for Acute Pancreatitis.

Shore ER, Awais M, Kershaw NM, Gibson RR, Pandalaneni S, Latawiec D, Wen L, Javed MA, Criddle DN, Berry N, O'Neill PM, Lian LY, Sutton R.

J Med Chem. 2016 Mar 24;59(6):2596-611

IV. Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca^{2+} dependent mitochondrial dysfunction and acute pancreatitis.

Huang W, Booth DM, Cane MC, Chvanov M, Javed MA, Elliott VL, Armstrong JA, Dingsdale H, Cash N, Li Y, Greenhalf W, Mukherjee R, Kaphalia BS, Jaffar M, Petersen OH, Tepikin AV, Sutton R, Criddle DN.

Gut. 2014 Aug;63(8):1313-24

V. Book chapter: Specific treatment for acute pancreatitis (chapter 9)

Title - Pancreatitis: Medical and surgical management
Li Wen, Muhammad A. Javed, Kiran Altaf, Peter Szatmary and Robert Sutton.

Edited by David B. Adams. Published Online: 11 FEB 2017
DOI: 10.1002/9781118924907.ch9

BIBLIOGRAPHY

Abita, J. P., M. Delaage and M. Lazdunski (1969). "The mechanism of activation of trypsinogen. The role of the four N-terminal aspartyl residues." Eur J Biochem **8**(3): 314-324.

Acosta, J. M. and C. L. Ledesma (1974). "Gallstone migration as a cause of acute pancreatitis." N Engl J Med **290**(9): 484-487.

Afghani, E., S. J. Pandol, T. Shimosegawa, R. Sutton, B. U. Wu, S. S. Vege, F. Gorelick, M. Hirota, J. Windsor, S. K. Lo, M. L. Freeman, M. M. Lerch, Y. Tsuji, G. Y. Melmed, W. Wassef and J. Mayerle (2015). "Acute Pancreatitis-Progress and Challenges: A Report on an International Symposium." Pancreas **44**(8): 1195-1210.

Algul, H., Y. Tando, M. Beil, C. K. Weber, C. Von Weyhern, G. Schneider, G. Adler and R. M. Schmid (2002). "Different modes of NF-kappaB/Rel activation in pancreatic lobules." Am J Physiol Gastrointest Liver Physiol **283**(2): G270-281.

Alhan, E., N. I. Kalyoncu, C. Ercin and B. V. Kural (2004). "Effects of the celecoxib on the acute necrotizing pancreatitis in rats." Inflammation **28**(5): 303-309.

Alhan, E., S. Turkyilmaz, C. Ercin and B. V. Kural (2006). "Effects of lazaroid U-74389G on acute necrotizing pancreatitis in rats." Eur Surg Res **38**(2): 70-75.

Alhan, E., A. Usta, S. Turkyilmaz, B. V. Kural and C. Ercin (2015). "Effects of glutamine alone on the acute necrotizing pancreatitis in rats." J Surg Res **193**(1): 161-167.

Alsfasser, G., A. L. Warshaw, S. P. Thayer, B. Antoniu, M. Laposata, K. B. Lewandrowski and C. Fernandez-del Castillo (2006). "Decreased inflammation and improved survival with recombinant human activated protein

C treatment in experimental acute pancreatitis." Arch Surg **141**(7): 670-676; discussion 676-677.

Andriulli, A., L. Solmi, S. Loperfido, P. Leo, V. Festa, A. Belmonte, F. Spirito, M. Silla, G. Forte, V. Terruzzi, G. Marenco, E. Ciliberto, A. Sabatino, F. Monica, M. R. Magnolia and F. Perri (2004). "Prophylaxis of ERCP-related pancreatitis: a randomized, controlled trial of somatostatin and gabexate mesylate." Clin Gastroenterol Hepatol **2**(8): 713-718.

Anholt, R. R., P. L. Pedersen, E. B. De Souza and S. H. Snyder (1986). "The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane." J Biol Chem **261**(2): 576-583.

Arevalo-Rodriguez, M., X. Wu, S. D. Hanes and J. Heitman (2004). "Prolyl isomerases in yeast." Front Biosci **9**: 2420-2446.

Argaud, L., O. Gateau-Roesch, D. Muntean, L. Chalabreysse, J. Loufouat, D. Robert and M. Ovize (2005). "Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury." J Mol Cell Cardiol **38**(2): 367-374.

Ashburn, T. T. and K. B. Thor (2004). "Drug repositioning: identifying and developing new uses for existing drugs." Nat Rev Drug Discov **3**(8): 673-683.

Ashby, M. C. and A. V. Tepikin (2002). "Polarized calcium and calmodulin signaling in secretory epithelia." Physiol Rev **82**(3): 701-734.

Atar, D., H. Arheden, A. Berdeaux, J. L. Bonnet, M. Carlsson, P. Clemmensen, V. Cuvier, N. Danchin, J. L. Dubois-Rande, H. Engblom, D. Erlinge, H. Firat, S. Halvorsen, H. S. Hansen, W. Hauke, E. Heiberg, S. Koul, A. I. Larsen, P. Le Corvoisier, J. E. Nordrehaug, F. Paganelli, R. M. Pruss, H. Rousseau, S. Schaller, G. Sonou, V. Tuseth, J. Veys, E. Vicaut and S. E. Jensen (2015). "Effect of intravenous TRO40303 as an adjunct to primary

percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results." Eur Heart J **36**(2): 112-119.

Babu, B. I., T. Genovese, E. Mazzon, L. Riccardi, I. Paterniti, M. Galuppo, C. Crisafulli, A. K. Siriwardena and S. Cuzzocrea (2012). "Recombinant human activated protein C (Xigris) attenuates murine cerulein-induced acute pancreatitis via regulation of nuclear factor kappaB and apoptotic pathways." Pancreas **41**(4): 619-628.

Baines, C. P. (2009). "The mitochondrial permeability transition pore and ischemia-reperfusion injury." Basic Res Cardiol **104**(2): 181-188.

Baines, C. P., R. A. Kaiser, N. H. Purcell, N. S. Blair, H. Osinska, M. A. Hambleton, E. W. Brunskill, M. R. Sayen, R. A. Gottlieb, G. W. Dorn, J. Robbins and J. D. Molkentin (2005). "Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death." Nature **434**(7033): 658-662.

Balog, A., Z. Gyulai, L. G. Boros, G. Farkas, T. Takacs, J. Lonovics and Y. Mandi (2005). "Polymorphism of the TNF-alpha, HSP70-2, and CD14 genes increases susceptibility to severe acute pancreatitis." Pancreas **30**(2): e46-50.

Baltatzis, M., S. Jegatheeswaran, D. A. O'Reilly and A. K. Siriwardena (2016). "Antibiotic use in acute pancreatitis: Global overview of compliance with international guidelines." Pancreatology **16**(2): 189-193.

Banks, P. A., T. L. Bollen, C. Dervenis, H. G. Gooszen, C. D. Johnson, M. G. Sarr, G. G. Tsiotos, S. S. Vege and G. Acute Pancreatitis Classification Working (2013). "Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus." Gut **62**(1): 102-111.

Bansal, D., A. Bhalla, D. K. Bhasin, P. Pandhi, N. Sharma, S. Rana and S. Malhotra (2011). "Safety and efficacy of vitamin-based antioxidant therapy in patients with severe acute pancreatitis: a randomized controlled trial." Saudi J Gastroenterol **17**(3): 174-179.

Barkay, O., E. Niv, E. Santo, R. Bruck, A. Hallak and F. M. Konikoff (2008). "Low-dose heparin for the prevention of post-ERCP pancreatitis: a randomized placebo-controlled trial." Surg Endosc **22**(9): 1971-1976.

Berger, Z., R. Quera, J. Poniachik, D. Oksenberg and J. Guerrero (2001). "[heparin and insulin treatment of acute pancreatitis caused by hypertriglyceridemia. Experience of 5 cases]." Rev Med Chil **129**(12): 1373-1378.

Berling, R., S. Genell and K. Ohlsson (1994). "High-dose intraperitoneal aprotinin treatment of acute severe pancreatitis: a double-blind randomized multi-center trial." J Gastroenterol **29**(4): 479-485.

Bernardi, P., A. Krauskopf, E. Basso, V. Petronilli, E. Blachly-Dyson, F. Di Lisa and M. A. Forte (2006). "The mitochondrial permeability transition from in vitro artifact to disease target." FEBS J **273**(10): 2077-2099.

Bernardi, P., A. Rasola, M. Forte and G. Lippe (2015). "The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology." Physiol Rev **95**(4): 1111-1155.

Bhagat, L., V. P. Singh, R. K. Dawra and A. K. Saluja (2008). "Sodium arsenite induces heat shock protein 70 expression and protects against secretagogue-induced trypsinogen and NF-kappaB activation." J Cell Physiol **215**(1): 37-46.

Bhagat, L., V. P. Singh, A. M. Song, G. J. van Acker, S. Agrawal, M. L. Steer and A. K. Saluja (2002). "Thermal stress-induced HSP70 mediates

protection against intrapancreatic trypsinogen activation and acute pancreatitis in rats." Gastroenterology **122**(1): 156-165.

Bhatia, M., M. Brady, S. Shokuhi, S. Christmas, J. P. Neoptolemos and J. Slavin (2000). "Inflammatory mediators in acute pancreatitis." J Pathol **190**(2): 117-125.

Bhatia, M., J. P. Neoptolemos and J. Slavin (2001). "Inflammatory mediators as therapeutic targets in acute pancreatitis." Curr Opin Investig Drugs **2**(4): 496-501.

Bhatia, M., A. K. Saluja, B. Hofbauer, H. S. Lee, J. L. Frossard and M. L. Steer (1998). "The effects of neutrophil depletion on a completely noninvasive model of acute pancreatitis-associated lung injury." Int J Pancreatol **24**(2): 77-83.

Bhatia, V., V. Ahuja, S. K. Acharya and P. K. Garg (2011). "A randomized controlled trial of valdecoxib and glyceryl trinitrate for the prevention of post-ERCP pancreatitis." J Clin Gastroenterol **45**(2): 170-176.

Bockman, D. E., W. R. Boydston and I. Parsa (1983). "Architecture of human pancreas: implications for early changes in pancreatic disease." Gastroenterology **85**(1): 55-61.

Booth, D. M., J. A. Murphy, R. Mukherjee, M. Awais, J. P. Neoptolemos, O. V. Gerasimenko, A. V. Tepikin, O. H. Petersen, R. Sutton and D. N. Criddle (2011). "Reactive oxygen species induced by bile acid induce apoptosis and protect against necrosis in pancreatic acinar cells." Gastroenterology **140**(7): 2116-2125.

Borel, J. F. and Z. L. Kis (1991). "The discovery and development of cyclosporine (Sandimmune)." Transplant Proc **23**(2): 1867-1874.

Boyce, M. and J. Yuan (2006). "Cellular response to endoplasmic reticulum stress: a matter of life or death." Cell Death Differ **13**(3): 363-373.

Broekemeier, K. M., M. E. Dempsey and D. R. Pfeiffer (1989). "Cyclosporin A is a potent inhibitor of the inner membrane permeability transition in liver mitochondria." J Biol Chem **264**(14): 7826-7830.

Budzynska, A., T. Marek, A. Nowak, R. Kaczor and E. Nowakowska-Dulawa (2001). "A prospective, randomized, placebo-controlled trial of prednisone and allopurinol in the prevention of ERCP-induced pancreatitis." Endoscopy **33**(9): 766-772.

Bukowczan, J., J. Cieszkowski, Z. Warzecha, P. Ceranowicz, B. Kusnierz-Cabala, R. Tomaszewska and A. Dembinski (2016). "Therapeutic Effect of Obestatin in the Course of Cerulein-Induced Acute Pancreatitis." Pancreas **45**(5): 700-706.

Bukowczan, J., Z. Warzecha, P. Ceranowicz, B. Kusnierz-Cabala and R. Tomaszewska (2015). "Obestatin Accelerates the Recovery in the Course of Ischemia/Reperfusion-Induced Acute Pancreatitis in Rats." PLoS One **10**(7): e0134380.

Burdakov, D., O. H. Petersen and A. Verkhratsky (2005). "Intraluminal calcium as a primary regulator of endoplasmic reticulum function." Cell Calcium **38**(3-4): 303-310.

Busnardo, A. C., L. J. DiDio, R. T. Tidrick and N. R. Thomford (1983). "History of the pancreas." Am J Surg **146**(5): 539-550.

Buyukberber, M., M. C. Savas, C. Bagci, M. Koruk, M. T. Gulsen, E. Tutar, T. Bilgic and N. O. Ceylan (2009). "Therapeutic effect of caffeic acid phenethyl ester on cerulein-induced acute pancreatitis." World J Gastroenterol **15**(41): 5181-5185.

Cameron, J. L., D. Mehigan and G. D. Zuidema (1979). "Evaluation of atropine in acute pancreatitis." Surg Gynecol Obstet **148**(2): 206-208.

Cao, J. and Q. Liu (2013). "Protective effects of sivelestat in a caerulein-induced rat acute pancreatitis model." Inflammation **36**(6): 1348-1356.

Ceranowicz, P., A. Dembinski, Z. Warzecha, M. Dembinski, J. Cieszkowski, K. Rembisz, S. J. Konturek, B. Kusnierz-Cabala, R. Tomaszewska and W. W. Pawlik (2008). "Protective and therapeutic effect of heparin in acute pancreatitis." J Physiol Pharmacol **59 Suppl 4**: 103-125.

Ceyhan, G. O., A. K. Timm, F. Bergmann, A. Gunther, A. A. Aghdassi, I. E. Demir, J. Mayerle, M. Kern, M. M. Lerch, M. W. Buchler, H. Friess and P. Schemmer (2011). "Prophylactic glycine administration attenuates pancreatic damage and inflammation in experimental acute pancreatitis." Pancreatology **11**(1): 57-67.

Chappell, J. B. and A. R. Crofts (1965). "Calcium Ion Accumulation and Volume Changes of Isolated Liver Mitochondria. Calcium Ion-Induced Swelling." Biochem J **95**: 378-386.

Charan, J. and N. D. Kantharia (2013). "How to calculate sample size in animal studies?" J Pharmacol Pharmacother **4**(4): 303-306.

Chen, G. Y., R. W. Dai, H. Luo, W. H. Liu, T. Chen, N. Lin, T. Wang, G. D. Luo and L. J. Tang (2015). "Effect of percutaneous catheter drainage on pancreatic injury in rats with severe acute pancreatitis induced by sodium taurocholate." Pancreatology **15**(1): 71-77.

Chen, W., L. Feng, H. Nie and X. Zheng (2012). "Andrographolide induces autophagic cell death in human liver cancer cells through cyclophilin D-mediated mitochondrial permeability transition pore." Carcinogenesis **33**(11): 2190-2198.

Chen, Z. Q., Y. Q. Tang, Y. Zhang, Z. H. Jiang, E. Q. Mao, W. G. Zou, R. Q. Lei, T. Q. Han and S. D. Zhang (2004). "Adenoviral transfer of human interleukin-10 gene in lethal pancreatitis." World J Gastroenterol **10**(20): 3021-3025.

Choi, C. W., D. H. Kang, G. H. Kim, J. S. Eum, S. M. Lee, G. A. Song, D. U. Kim, I. D. Kim and M. Cho (2009). "Nafamostat mesylate in the prevention of post-ERCP pancreatitis and risk factors for post-ERCP pancreatitis." Gastrointest Endosc **69**(4): e11-18.

Christophe, J. (1994). "Pancreatic tumoral cell line AR42J: an amphicrine model." Am J Physiol **266**(6 Pt 1): G963-971.

Christophi, C., I. Millar, M. Nikfarjam, V. Muralidharan and C. Malcontenti-Wilson (2007). "Hyperbaric oxygen therapy for severe acute pancreatitis." J Gastroenterol Hepatol **22**(11): 2042-2046.

Clarke, S. J., G. P. McStay and A. P. Halestrap (2002). "Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A." J Biol Chem **277**(38): 34793-34799.

Cleary, J., K. M. Johnson, A. W. Opipari, Jr. and G. D. Glick (2007). "Inhibition of the mitochondrial F1F0-ATPase by ligands of the peripheral benzodiazepine receptor." Bioorg Med Chem Lett **17**(6): 1667-1670.

Clipstone, N. A. and G. R. Crabtree (1992). "Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation." Nature **357**(6380): 695-697.

Constanzo, L. (2006). Gastrointestinal physiology. BRS physiology 4th edition. L. Constanzo. Philadelphia, USA, Lippincott Williams and Wilkins.

Cote, G. A., D. Yadav, A. Slivka, R. H. Hawes, M. A. Anderson, F. R. Burton, R. E. Brand, P. A. Banks, M. D. Lewis, J. A. Disario, T. B. Gardner, A. Gelrud, S. T. Amann, J. Baillie, M. E. Money, M. O'Connell, D. C. Whitcomb, S. Sherman and G. North American Pancreatitis Study (2011). "Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis." Clin Gastroenterol Hepatol **9**(3): 266-273; quiz e227.

Criddle, D. N., D. M. Booth, R. Mukherjee, E. McLaughlin, G. M. Green, R. Sutton, O. H. Petersen and J. R. Reeve, Jr. (2009). "Cholecystokinin-58 and cholecystokinin-8 exhibit similar actions on calcium signaling, zymogen secretion, and cell fate in murine pancreatic acinar cells." Am J Physiol Gastrointest Liver Physiol **297**(6): G1085-1092.

Criddle, D. N., J. V. Gerasimenko, H. K. Baumgartner, M. Jaffar, S. Voronina, R. Sutton, O. H. Petersen and O. V. Gerasimenko (2007). "Calcium signalling and pancreatic cell death: apoptosis or necrosis?" Cell Death Differ **14**(7): 1285-1294.

Criddle, D. N., E. McLaughlin, J. A. Murphy, O. H. Petersen and R. Sutton (2007). "The pancreas misled: signals to pancreatitis." Pancreatology **7**(5-6): 436-446.

Criddle, D. N., J. Murphy, G. Fistetto, S. Barrow, A. V. Tepikin, J. P. Neoptolemos, R. Sutton and O. H. Petersen (2006). "Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis." Gastroenterology **130**(3): 781-793.

Criddle, D. N., M. G. Raraty, J. P. Neoptolemos, A. V. Tepikin, O. H. Petersen and R. Sutton (2004). "Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites." Proc Natl Acad Sci U S A **101**(29): 10738-10743.

Crompton, M., A. Costi and L. Hayat (1987). "Evidence for the presence of a reversible Ca2+-dependent pore activated by oxidative stress in heart mitochondria." Biochem J **245**(3): 915-918.

Crompton, M., H. Ellinger and A. Costi (1988). "Inhibition by cyclosporin A of a Ca2+-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress." Biochem J **255**(1): 357-360.

Culty, M., H. Li, N. Boujrad, H. Amri, B. Vidic, J. M. Bernassau, J. L. Reversat and V. Papadopoulos (1999). "In vitro studies on the role of the peripheral-type benzodiazepine receptor in steroidogenesis." J Steroid Biochem Mol Biol **69**(1-6): 123-130.

Cung, T. T., O. Morel, G. Cayla, G. Rioufol, D. Garcia-Dorado, D. Angoulvant, E. Bonnefoy-Cudraz, P. Guerin, M. Elbaz, N. Delarche, P. Coste, G. Vanzetto, M. Metge, J. F. Aupetit, B. Jouve, P. Motreff, C. Tron, J. N. Labeque, P. G. Steg, Y. Cottin, G. Range, J. Clerc, M. J. Claeys, P. Coussement, F. Prunier, F. Moulin, O. Roth, L. Belle, P. Dubois, P. Barragan, M. Gilard, C. Piot, P. Colin, F. De Poli, M. C. Morice, O. Ider, J. L. Dubois-Rande, T. Unterseeh, H. Le Breton, T. Beard, D. Blanchard, G. Grollier, V. Malquarti, P. Staat, A. Sudre, E. Elmer, M. J. Hansson, C. Bergerot, I. Boussaha, C. Jossan, G. Derumeaux, N. Mewton and M. Ovize (2015). "Cyclosporine before PCI in Patients with Acute Myocardial Infarction." N Engl J Med **373**(11): 1021-1031.

da Costa, D. W., L. M. Dijksman, S. A. Bouwense, N. J. Schepers, M. G. Besselink, H. C. van Santvoort, D. Boerma, H. G. Gooszen, M. G. Dijkgraaf and G. Dutch Pancreatitis Study (2016). "Cost-effectiveness of same-admission versus interval cholecystectomy after mild gallstone pancreatitis in the PONCHO trial." Br J Surg **103**(12): 1695-1703.

da Silva, I. R. and L. McWilliams (2013). "Moderate therapeutic hypothermia in a patient with acute pancreatitis: case report and review of the literature." Pancreas **42**(3): 544-545.

Davidson, S. M. and M. R. Duchen (2006). "Calcium microdomains and oxidative stress." Cell Calcium **40**(5-6): 561-574.

Davies, M. G. and P. O. Hagen (1997). "Systemic inflammatory response syndrome." Br J Surg **84**(7): 920-935.

Davis, T. L., J. R. Walker, V. Campagna-Slater, P. J. Finerty, R. Paramanathan, G. Bernstein, F. MacKenzie, W. Tempel, H. Ouyang, W. H. Lee, E. Z. Eisenmesser and S. Dhe-Paganon (2010). "Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases." PLoS Biol **8**(7): e1000439.

Dawra, R., Y. S. Ku, R. Sharif, D. Dhaulakhandi, P. Phillips, V. Dudeja and A. K. Saluja (2008). "An improved method for extracting myeloperoxidase and determining its activity in the pancreas and lungs during pancreatitis." Pancreas **37**(1): 62-68.

Dawra, R., R. P. Sah, V. Dudeja, L. Rishi, R. Talukdar, P. Garg and A. K. Saluja (2011). "Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis." Gastroenterology **141**(6): 2210-2217 e2212.

De Palma, G. D. and C. Catanzano (1999). "Use of corticosteriods in the prevention of post-ERCP pancreatitis: results of a controlled prospective study." Am J Gastroenterol **94**(4): 982-985.

de Tassigny, A., R. Assaly, S. Schaller, R. M. Pruss, A. Berdeaux and D. Morin (2013). "Mitochondrial translocator protein (TSPO) ligands prevent doxorubicin-induced mechanical dysfunction and cell death in isolated cardiomyocytes." Mitochondrion **13**(6): 688-697.

Debas, H. T., R. J. Hancock, P. Soon-Shiong, H. A. Smythe and M. M. Cassim (1980). "Glucagon therapy in acute pancreatitis: prospective randomized double-blind study." Can J Surg **23**(6): 578-580.

Dembinski, A., Z. Warzecha, P. Ceranowicz, J. Stachura, R. Tomaszewska, S. J. Konturek, R. Sendur, M. Dembinski and W. W. Pawlik (2001). "Pancreatic damage and regeneration in the course of ischemia-reperfusion induced pancreatitis in rats." J Physiol Pharmacol **52**(2): 221-235.

Deng, W. H., C. Chen, W. X. Wang, J. Yu, J. Y. Li and L. Liu (2011). "Effects of ORP150 on appearance and function of pancreatic beta cells following acute necrotizing pancreatitis." Pathol Res Pract **207**(6): 370-376.

Derler, I., R. Schindl, R. Fritsch, P. Heftberger, M. C. Riedl, M. Begg, D. House and C. Romanin (2013). "The action of selective CRAC channel blockers is affected by the Orai pore geometry." Cell Calcium **53**(2): 139-151.

Devalaraja-Narashimha, K., A. M. Diener and B. J. Padanilam (2009). "Cyclophilin D gene ablation protects mice from ischemic renal injury." Am J Physiol Renal Physiol **297**(3): F749-759.

Deviere, J., O. Le Moine, J. L. Van Laethem, P. Eisendrath, A. Ghilain, N. Severs and M. Cohard (2001). "Interleukin 10 reduces the incidence of pancreatitis after therapeutic endoscopic retrograde cholangiopancreatography." Gastroenterology **120**(2): 498-505.

Ding, S. P., J. C. Li and C. Jin (2003). "A mouse model of severe acute pancreatitis induced with caerulein and lipopolysaccharide." World J Gastroenterol **9**(3): 584-589.

Dobronte, Z., Z. Szepes, F. Izbeki, J. Gervain, L. Lakatos, G. Pecsi, M. Ihasz, L. Lakner, E. Toldy and L. Czako (2014). "Is rectal indomethacin effective in preventing of post-endoscopic retrograde

cholangiopancreatography pancreatitis?" World J Gastroenterol **20**(29): 10151-10157.

Du, H., L. Guo, F. Fang, D. Chen, A. A. Sosunov, G. M. McKhann, Y. Yan, C. Wang, H. Zhang, J. D. Molkentin, F. J. Gunn-Moore, J. P. Vonsattel, O. Arancio, J. X. Chen and S. D. Yan (2008). "Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease." Nat Med **14**(10): 1097-1105.

Du, W. D., Z. R. Yuan, J. Sun, J. X. Tang, A. Q. Cheng, D. M. Shen, C. J. Huang, X. H. Song, X. F. Yu and S. B. Zheng (2003). "Therapeutic efficacy of high-dose vitamin C on acute pancreatitis and its potential mechanisms." World J Gastroenterol **9**(11): 2565-2569.

Dumot, J. A., D. L. Conwell, J. B. O'Connor, D. R. Ferguson, J. J. Vargo, D. S. Barnes, S. S. Shay, M. J. Sterling, K. S. Horth, K. Issa, J. L. Ponsky and G. Zuccaro (1998). "Pretreatment with methylprednisolone to prevent ERCP-induced pancreatitis: a randomized, multicenter, placebo-controlled clinical trial." Am J Gastroenterol **93**(1): 61-65.

Dumot, J. A., D. L. Conwell, G. Zuccaro, Jr., J. J. Vargo, S. S. Shay, K. A. Easley and J. L. Ponsky (2001). "A randomized, double blind study of interleukin 10 for the prevention of ERCP-induced pancreatitis." Am J Gastroenterol **96**(7): 2098-2102.

Dunsmore, C. J., K. J. Malone, K. R. Bailey, M. A. Wear, H. Florance, S. Shirran, P. E. Barran, A. P. Page, M. D. Walkinshaw and N. J. Turner (2011). "Design and synthesis of conformationally constrained cyclophilin inhibitors showing a cyclosporin-A phenotype in C. elegans." Chembiochem **12**(5): 802-810.

Duran-Bedolla, J., M. A. Montes de Oca-Sandoval, V. Saldana-Navor, J. A. Villalobos-Silva, M. C. Rodriguez and S. Rivas-Arancibia (2014).

"Sepsis, mitochondrial failure and multiple organ dysfunction." Clin Invest Med **37**(2): E58-69.

Easterbrook, P. J., J. A. Berlin, R. Gopalan and D. R. Matthews (1991). "Publication bias in clinical research." Lancet **337**(8746): 867-872.

Ellis, M. P., J. J. French and R. M. Charnley (2009). "Acute pancreatitis and the influence of socioeconomic deprivation." Br J Surg **96**(1): 74-80.

Elmunzer, B. J., J. M. Scheiman, G. A. Lehman, A. Chak, P. Mosler, P. D. Higgins, R. A. Hayward, J. Romagnuolo, G. H. Elta, S. Sherman, A. K. Waljee, A. Repaka, M. R. Atkinson, G. A. Cote, R. S. Kwon, L. McHenry, C. R. Piraka, E. J. Wamsteker, J. L. Watkins, S. J. Korsnes, S. E. Schmidt, S. M. Turner, S. Nicholson, E. L. Fogel and U. S. C. f. O. R. i. Endoscopy (2012). "A randomized trial of rectal indomethacin to prevent post-ERCP pancreatitis." N Engl J Med **366**(15): 1414-1422.

Elrod, J. W. and J. D. Molkentin (2013). "Physiologic functions of cyclophilin D and the mitochondrial permeability transition pore." Circ J **77**(5): 1111-1122.

Essardas Daryanani, H., F. J. Santolaria Fernandez, C. E. Gonzalez Reimers, J. A. Jorge Hernandez, N. Batista Lopez, I. Gonzalez Martin and L. Hernandez Nieto (1983). "[Acute pancreatitis: comparative therapeutic study among cimetidine, oxyphenonium and simple nasogastric aspiration]." Med Clin (Barc) **81**(1): 4-6.

Ethridge, R. T., R. A. Ehlers, M. R. Hellmich, S. Rajaraman and B. M. Evers (2000). "Acute pancreatitis results in induction of heat shock proteins 70 and 27 and heat shock factor-1." Pancreas **21**(3): 248-256.

Fakharnia, F., F. Khodagholi, L. Dargahi and A. Ahmadiani (2017). "Prevention of Cyclophilin D-Mediated mPTP Opening Using Cyclosporine-A

Alleviates the Elevation of Necroptosis, Autophagy and Apoptosis-Related Markers Following Global Cerebral Ischemia-Reperfusion." J Mol Neurosci **61**(1): 52-60.

Fazio, E. N., G. E. Dimattia, S. A. Chadi, K. D. Kernohan and C. L. Pin (2011). "Stanniocalcin 2 alters PERK signalling and reduces cellular injury during cerulein induced pancreatitis in mice." BMC Cell Biol **12**: 17.

Fink, M. P. (2014). "Animal models of sepsis." Virulence **5**(1): 143-153.

Fischer, G., H. Bang and C. Mech (1984). "[Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides]." Biomed Biochim Acta **43**(10): 1101-1111.

Fischer, G., B. Wittmann-Liebold, K. Lang, T. Kiefhaber and F. X. Schmid (1989). "Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins." Nature **337**(6206): 476-478.

FITZ , R. H. (1889). "Acute Pancreatitis." The Boston Medical and Surgical Journal **120**(8): 181-187.

Flint, R. S. and J. A. Windsor (2003). "The role of the intestine in the pathophysiology and management of severe acute pancreatitis." HPB (Oxford) **5**(2): 69-85.

Fluhr, G., J. Mayerle, E. Weber, A. Aghdassi, P. Simon, T. Gress, T. Seufferlein, J. Mossner, A. Stallmach, T. Rosch, M. Muller, B. Siegmund, P. Buchner-Steudel, I. Zuber-Jerger, M. Kantowski, A. Hoffmeister, J. Rosendahl, T. Linhart, J. Maul, L. Czako, P. Hegyi, M. Kraft, G. Engel, T. Kohlmann, A. Glitsch, T. Pickartz, C. Budde, C. Nitsche, K. Storck and M. M. Lerch (2013). "Pre-study protocol MagPEP: a multicentre randomized controlled trial of magnesium sulphate in the prevention of post-ERCP pancreatitis." BMC Gastroenterol **13**: 11.

Foitzik, T., B. Forgacs, E. Ryschich, H. Hotz, M. M. Gebhardt, H. J. Buhr and E. Klar (1998). "Effect of different immunosuppressive agents on acute pancreatitis: a comparative study in an improved animal model." Transplantation **65**(8): 1030-1036.

Foitzik, T., H. G. Hotz, B. Hotz, F. Wittig and H. J. Buhr (2003). "Selective inhibition of cyclooxygenase-2 (COX-2) reduces prostaglandin E2 production and attenuates systemic disease sequelae in experimental pancreatitis." Hepatogastroenterology **50**(52): 1159-1162.

Folch-Puy, E., S. Granell, J. L. Iovanna, M. Barthet and D. Closa (2006). "Peroxisome proliferator-activated receptor gamma agonist reduces the severity of post-ERCP pancreatitis in rats." World J Gastroenterol **12**(40): 6458-6463.

Fonai, F., J. K. Priber, P. B. Jakus, N. Kalman, C. Antus, E. Pollak, G. Karsai, L. Tretter, B. Sumegi and B. Veres (2015). "Lack of cyclophilin D protects against the development of acute lung injury in endotoxemia." Biochim Biophys Acta **1852**(12): 2563-2573.

Fontaine, E., O. Eriksson, F. Ichas and P. Bernardi (1998). "Regulation of the permeability transition pore in skeletal muscle mitochondria. Modulation By electron flow through the respiratory chain complex i." J Biol Chem **273**(20): 12662-12668.

Forsmark, C. E., S. S. Vege and C. M. Wilcox (2016). "Acute Pancreatitis." N Engl J Med **375**(20): 1972-1981.

Freise, J., F. W. Schmidt, P. Magerstedt and K. Schmid (1985). "Gabexate mesilate and camostate: new inhibitors of phospholipase A2 and their influence on the alpha-amylase activity in serum of patients with acute pancreatitis." Clin Biochem **18**(4): 224-229.

Frossard, J. L., C. M. Pastor and A. Hadengue (2001). "Effect of hyperthermia on NF-kappaB binding activity in cerulein-induced acute pancreatitis." Am J Physiol Gastrointest Liver Physiol **280**(6): G1157-1162.

Frossard, J. L., A. Saluja, L. Bhagat, H. S. Lee, M. Bhatia, B. Hofbauer and M. L. Steer (1999). "The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury." Gastroenterology **116**(3): 694-701.

Fu, S., S. M. Watkins and G. S. Hotamisligil (2012). "The role of endoplasmic reticulum in hepatic lipid homeostasis and stress signaling." Cell Metab **15**(5): 623-634.

Fujimoto, K., Y. Chen, K. S. Polonsky and G. W. Dorn, 2nd (2010). "Targeting cyclophilin D and the mitochondrial permeability transition enhances beta-cell survival and prevents diabetes in Pdx1 deficiency." Proc Natl Acad Sci U S A **107**(22): 10214-10219.

Futatsugi, A., T. Nakamura, M. K. Yamada, E. Ebisui, K. Nakamura, K. Uchida, T. Kitaguchi, H. Takahashi-Iwanaga, T. Noda, J. Aruga and K. Mikoshiba (2005). "IP3 receptor types 2 and 3 mediate exocrine secretion underlying energy metabolism." Science **309**(5744): 2232-2234.

Gaiser, S., J. Daniluk, Y. Liu, L. Tsou, J. Chu, W. Lee, D. S. Longnecker, C. D. Logsdon and B. Ji (2011). "Intracellular activation of trypsinogen in transgenic mice induces acute but not chronic pancreatitis." Gut **60**(10): 1379-1388.

Gavish, M., I. Bachman, R. Shoukrun, Y. Katz, L. Veenman, G. Weisinger and A. Weizman (1999). "Enigma of the peripheral benzodiazepine receptor." Pharmacol Rev **51**(4): 629-650.

Gerasimenko, J. V., O. Gryshchenko, P. E. Ferdek, E. Stapleton, T. O. Hebert, S. Bychkova, S. Peng, M. Begg, O. V. Gerasimenko and O. H.

Petersen (2013). "Ca2+ release-activated Ca2+ channel blockade as a potential tool in antipancreatitis therapy." Proc Natl Acad Sci U S A **110**(32): 13186-13191.

Gerasimenko, J. V., G. Lur, M. W. Sherwood, E. Ebisui, A. V. Tepikin, K. Mikoshiba, O. V. Gerasimenko and O. H. Petersen (2009). "Pancreatic protease activation by alcohol metabolite depends on Ca2+ release via acid store IP3 receptors." Proc Natl Acad Sci U S A **106**(26): 10758-10763.

Gineste, C., A. Hernandez, N. Ivarsson, A. J. Cheng, K. Naess, R. Wibom, N. Lesko, H. Bruhn, A. Wedell, C. Freyer, S. J. Zhang, M. Carlstrom, J. T. Lanner, D. C. Andersson, J. D. Bruton, A. Wredenberg and H. Westerblad (2015). "Cyclophilin D, a target for counteracting skeletal muscle dysfunction in mitochondrial myopathy." Hum Mol Genet **24**(23): 6580-6587.

Giorgio, V., E. Bisetto, M. E. Soriano, F. Dabbeni-Sala, E. Basso, V. Petronilli, M. A. Forte, P. Bernardi and G. Lippe (2009). "Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex." J Biol Chem **284**(49): 33982-33988.

Giorgio, V., S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G. D. Glick, V. Petronilli, M. Zoratti, I. Szabo, G. Lippe and P. Bernardi (2013). "Dimers of mitochondrial ATP synthase form the permeability transition pore." Proc Natl Acad Sci U S A **110**(15): 5887-5892.

Gloor, B., W. Uhl, O. Tcholakov, A. Roggo, C. A. Muller, M. Worni and M. W. Buchler (2001). "Hydrocortisone treatment of early SIRS in acute experimental pancreatitis." Dig Dis Sci **46**(10): 2154-2161.

Gomez, L., H. Thibault, A. Gharib, J. M. Dumont, G. Vuagniaux, P. Scalfaro, G. Derumeaux and M. Ovize (2007). "Inhibition of mitochondrial permeability transition improves functional recovery and reduces mortality following acute myocardial infarction in mice." Am J Physiol Heart Circ Physiol **293**(3): H1654-1661.

Gorelick, F. S. and M. M. Lerch (2017). "Do Animal Models of Acute Pancreatitis Reproduce Human Disease?" Cell Mol Gastroenterol Hepatol **4**(2): 251-262.

Gothel, S. F. and M. A. Marahiel (1999). "Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts." Cell Mol Life Sci **55**(3): 423-436.

Grady, T., P. Liang, S. A. Ernst and C. D. Logsdon (1997). "Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis." Gastroenterology **113**(6): 1966-1975.

Green, D. R. and B. Van Houten (2011). "SnapShot: Mitochondrial quality control." Cell **147**(4): 950, 950 e951.

Grewal, H. P., M. Kotb, A. M. el Din, M. Ohman, A. Salem, L. Gaber and A. O. Gaber (1994). "Induction of tumor necrosis factor in severe acute pancreatitis and its subsequent reduction after hepatic passage." Surgery **115**(2): 213-221.

Griffiths, E. J. and A. P. Halestrap (1995). "Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion." Biochem J **307 (Pt 1)**: 93-98.

Guichou, J. F., J. Viaud, C. Mettling, G. Subra, Y. L. Lin and A. Chavanieu (2006). "Structure-based design, synthesis, and biological evaluation of novel inhibitors of human cyclophilin A." J Med Chem **49**(3): 900-910.

Gukovskaya, A. S. and I. Gukovsky (2012). "Autophagy and pancreatitis." Am J Physiol Gastrointest Liver Physiol **303**(9): G993-G1003.

Gukovskaya, A. S., I. Gukovsky, V. Zaninovic, M. Song, D. Sandoval, S. Gukovsky and S. J. Pandol (1997). "Pancreatic acinar cells

produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis." J Clin Invest **100**(7): 1853-1862.

Gukovskaya, A. S., M. Mouria, I. Gukovsky, C. N. Reyes, V. N. Kasho, L. D. Faller and S. J. Pandol (2002). "Ethanol metabolism and transcription factor activation in pancreatic acinar cells in rats." Gastroenterology **122**(1): 106-118.

Gukovsky, I., N. Li, J. Todoric, A. Gukovskaya and M. Karin (2013). "Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer." Gastroenterology **144**(6): 1199-1209 e1194.

Gunjaca, I., J. Zunic, M. Gunjaca and Z. Kovac (2012). "Circulating cytokine levels in acute pancreatitis-model of SIRS/CARS can help in the clinical assessment of disease severity." Inflammation **35**(2): 758-763.

Gunter, T. E. and D. R. Pfeiffer (1990). "Mechanisms by which mitochondria transport calcium." Am J Physiol **258**(5 Pt 1): C755-786.

Halangk, W., M. M. Lerch, B. Brandt-Nedelev, W. Roth, M. Ruthenbuerger, T. Reinheckel, W. Domschke, H. Lippert, C. Peters and J. Deussing (2000). "Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis." J Clin Invest **106**(6): 773-781.

Halestrap, A. P. (2010). "A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection." Biochem Soc Trans **38**(4): 841-860.

Halestrap, A. P. and A. M. Davidson (1990). "Inhibition of Ca2(+)-induced large-amplitude swelling of liver and heart mitochondria by cyclosporin is probably caused by the inhibitor binding to mitochondrial-matrix peptidyl-prolyl cis-trans isomerase and preventing it interacting with the adenine nucleotide translocase." Biochem J **268**(1): 153-160.

Handschumacher, R. E., M. W. Harding, J. Rice, R. J. Drugge and D. W. Speicher (1984). "Cyclophilin: a specific cytosolic binding protein for cyclosporin A." Science **226**(4674): 544-547.

Hartley, T., M. Siva, E. Lai, T. Teodoro, L. Zhang and A. Volchuk (2010). "Endoplasmic reticulum stress response in an INS-1 pancreatic beta-cell line with inducible expression of a folding-deficient proinsulin." BMC Cell Biol **11**: 59.

Hatao, M. (1969). "On the etiology and pathophysiology of acute pancreatitis, with special reference to participation of phospholipase A." Nihon Geka Hokan **38**(1): 76-106.

Hay, M., D. W. Thomas, J. L. Craighead, C. Economides and J. Rosenthal (2014). "Clinical development success rates for investigational drugs." Nat Biotechnol **32**(1): 40-51.

He, Q., Q. Wang, C. Yuan and Y. Wang (2017). "Downregulation of miR-7116-5p in microglia by MPP+ sensitizes TNF-alpha production to induce dopaminergic neuron damage." Glia.

Hetz, C. (2012). "The unfolded protein response: controlling cell fate decisions under ER stress and beyond." Nat Rev Mol Cell Biol **13**(2): 89-102.

Hietaranta, A. J., V. P. Singh, L. Bhagat, G. J. van Acker, A. M. Song, A. Mykoniatis, M. L. Steer and A. K. Saluja (2001). "Water immersion stress prevents caerulein-induced pancreatic acinar cell nf-kappa b activation by attenuating caerulein-induced intracellular Ca2+ changes." J Biol Chem **276**(22): 18742-18747.

Hirano, T. (1994). "Protective effect of gabexate mesilate (FOY) against pancreatic injuries induced by ethanol in rats." Nihon Geka Hokan **63**(1): 10-20.

Ho, Y. P., C. T. Chiu, I. S. Sheen, S. C. Tseng, P. C. Lai, S. Y. Ho, W. T. Chen, T. N. Lin and C. Y. Lin (2011). "Tumor necrosis factor-alpha and interleukin-10 contribute to immunoparalysis in patients with acute pancreatitis." Hum Immunol **72**(1): 18-23.

Hong, S., B. Qiwen, J. Ying, A. Wei and T. Chaoyang (2011). "Body mass index and the risk and prognosis of acute pancreatitis: a meta-analysis." Eur J Gastroenterol Hepatol **23**(12): 1136-1143.

Honkoop, P., H. R. Scholte, R. A. de Man and S. W. Schalm (1997). "Mitochondrial injury. Lessons from the fialuridine trial." Drug Saf **17**(1): 1-7.

Hopkins, S. and P. Gallay (2012). "Cyclophilin inhibitors: an emerging class of therapeutics for the treatment of chronic hepatitis C infection." Viruses **4**(11): 2558-2577.

Hotamisligil, G. S. (2010). "Endoplasmic reticulum stress and the inflammatory basis of metabolic disease." Cell **140**(6): 900-917.

Huang, H., Y. Liu, J. Daniluk, S. Gaiser, J. Chu, H. Wang, Z. S. Li, C. D. Logsdon and B. Ji (2013). "Activation of nuclear factor-kappaB in acinar cells increases the severity of pancreatitis in mice." Gastroenterology **144**(1): 202-210.

Huang, L. and J. Cao (2014). "The protective effects of Shen-Fu injection on experimental acute pancreatitis in a rat model." Oxid Med Cell Longev **2014**: 248786.

Huang, W., D. M. Booth, M. C. Cane, M. Chvanov, M. A. Javed, V. L. Elliott, J. A. Armstrong, H. Dingsdale, N. Cash, Y. Li, W. Greenhalf, R. Mukherjee, B. S. Kaphalia, M. Jaffar, O. H. Petersen, A. V. Tepikin, R. Sutton and D. N. Criddle (2014). "Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca2+-dependent mitochondrial dysfunction and acute pancreatitis." Gut **63**(8): 1313-1324.

Huang, W., M. C. Cane, R. Mukherjee, P. Szatmary, X. Zhang, V. Elliott, Y. Ouyang, M. Chvanov, D. Latawiec, L. Wen, D. M. Booth, A. C. Haynes, O. H. Petersen, A. V. Tepikin, D. N. Criddle and R. Sutton (2015). "Caffeine protects against experimental acute pancreatitis by inhibition of inositol 1,4,5-trisphosphate receptor-mediated Ca2+ release." Gut.

Huang, W., N. Cash, L. Wen, P. Szatmary, R. Mukherjee, J. Armstrong, M. Chvanov, A. V. Tepikin, M. P. Murphy, R. Sutton and D. N. Criddle (2015). "Effects of the mitochondria-targeted antioxidant mitoquinone in murine acute pancreatitis." Mediators Inflamm **2015**: 901780.

Hunter, D. R. and R. A. Haworth (1979). "The Ca2+-induced membrane transition in mitochondria. I. The protective mechanisms." Arch Biochem Biophys **195**(2): 453-459.

Hunter, D. R., R. A. Haworth and J. H. Southard (1976). "Relationship between configuration, function, and permeability in calcium-treated mitochondria." J Biol Chem **251**(16): 5069-5077.

Ino, Y., Y. Arita, T. Akashi, T. Kimura, H. Igarashi, T. Oono, M. Furukawa, K. Kawabe, K. Ogoshi, J. Ouchi, T. Miyahara, R. Takayanagi and T. Ito (2008). "Continuous regional arterial infusion therapy with gabexate mesilate for severe acute pancreatitis." World J Gastroenterol **14**(41): 6382-6387.

Ionescu-Tirgoviste, C., P. A. Gagniuc, E. Gubceac, L. Mardare, I. Popescu, S. Dima and M. Militaru (2015). "A 3D map of the islet routes throughout the healthy human pancreas." Sci Rep **5**: 14634.

Itaba, S., K. Nakamura, A. Aso, S. Tokunaga, H. Akiho, E. Ihara, Y. Iboshi, T. Iwasa, K. Akahoshi, T. Ito and R. Takayanagi (2013). "Prospective, randomized, double-blind, placebo-controlled trial of ulinastatin for prevention of hyperenzymemia after double balloon endoscopy via the antegrade approach." Dig Endosc **25**(4): 421-427.

Ito, T., T. Kimura, H. Yamaguchi, M. Kinjo, T. Sumii, I. Nakano and H. Nawata (1993). "Acute pancreatitis induced by cyclosporin A under stimulation of pancreas by caerulein." Pancreas **8**(6): 693-699.

Izzo, V., J. M. Bravo-San Pedro, V. Sica, G. Kroemer and L. Galluzzi (2016). "Mitochondrial Permeability Transition: New Findings and Persisting Uncertainties." Trends Cell Biol **26**(9): 655-667.

Jacquier-Sarlin, M. R., K. Fuller, A. T. Dinh-Xuan, M. J. Richard and B. S. Polla (1994). "Protective effects of hsp70 in inflammation." Experientia **50**(11-12): 1031-1038.

Jadad, A. R., R. A. Moore, D. Carroll, C. Jenkinson, D. J. Reynolds, D. J. Gavaghan and H. J. McQuay (1996). "Assessing the quality of reports of randomized clinical trials: is blinding necessary?" Control Clin Trials **17**(1): 1-12.

Javadov, S., S. Jang, R. Parodi-Rullan, Z. Khuchua and A. V. Kuznetsov (2017). "Mitochondrial permeability transition in cardiac ischemia-reperfusion: whether cyclophilin D is a viable target for cardioprotection?" Cell Mol Life Sci.

Javed, M. A., L. Wen, M. Awais, D. Latawiec, W. Huang, M. Chvanov, S. Schaller, T. Bordet, M. Michaud, R. Pruss, A. Tepikin, D. Criddle and R. Sutton (2018). "TRO40303 Ameliorates Alcohol-Induced Pancreatitis Through Reduction of Fatty Acid Ethyl Ester-Induced Mitochondrial Injury and Necrotic Cell Death." Pancreas **47**(1): 18-24.

Johnson, C. D., A. N. Kingsnorth, C. W. Imrie, M. J. McMahon, J. P. Neoptolemos, C. McKay, S. K. Toh, P. Skaife, P. C. Leeder, P. Wilson, M. Larvin and L. D. Curtis (2001). "Double blind, randomised, placebo controlled study of a platelet activating factor antagonist, lexipafant, in the treatment and prevention of organ failure in predicted severe acute pancreatitis." Gut **48**(1): 62-69.

Jonas, E. A., G. A. Porter, Jr., G. Beutner, N. Mnatsakanyan and K. N. Alavian (2015). "Cell death disguised: The mitochondrial permeability transition pore as the c-subunit of the F(1)F(O) ATP synthase." Pharmacol Res **99**: 382-392.

Jowell, P. S., M. S. Branch, S. H. Fein, E. D. Purich, R. Kilaru, G. Robuck, P. d'Almada and J. Baillie (2011). "Intravenous synthetic secretin reduces the incidence of pancreatitis induced by endoscopic retrograde cholangiopancreatography." Pancreas **40**(4): 533-539.

Kajitani, K., M. Fujihashi, Y. Kobayashi, S. Shimizu, Y. Tsujimoto and K. Miki (2008). "Crystal structure of human cyclophilin D in complex with its inhibitor, cyclosporin A at 0.96-A resolution." Proteins **70**(4): 1635-1639.

Kang, R., M. T. Lotze, H. J. Zeh, T. R. Billiar and D. Tang (2014). "Cell death and DAMPs in acute pancreatitis." Mol Med **20**: 466-477.

Kapetanos, D., D. Christodoulou, O. Chatzizisi, D. Sigounas, K. Vasiliou, E. Stavropoulou, E. Katodritou, G. Kokozidis, G. Kiriazis, G. Kitis and E. Tsianos (2009). "Randomized study of the effect of pentoxifylline or octreotide on serum levels of TNF-alpha and IL-6 after endoscopic retrograde cholangiopancreatography." Eur J Gastroenterol Hepatol **21**(5): 529-533.

Katsinelos, P., J. Kountouras, J. Chatzis, K. Christodoulou, G. Paroutoglou, K. Mimidis, A. Beltsis and C. Zavos (2005). "High-dose allopurinol for prevention of post-ERCP pancreatitis: a prospective randomized double-blind controlled trial." Gastrointest Endosc **61**(3): 407-415.

Katsinelos, P., J. Kountouras, G. Paroutoglou, A. Beltsis, K. Mimidis and C. Zavos (2005). "Intravenous N-acetylcysteine does not prevent post-ERCP pancreatitis." Gastrointest Endosc **62**(1): 105-111.

Kilkenny, C., W. J. Browne, I. C. Cuthill, M. Emerson and D. G. Altman (2010). "Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research." PLoS Biol **8**(6): e1000412.

Kingsnorth, A. N., S. W. Galloway and L. J. Formela (1995). "Randomized, double-blind phase II trial of Lexipafant, a platelet-activating factor antagonist, in human acute pancreatitis." Br J Surg **82**(10): 1414-1420.

Kirichok, Y., G. Krapivinsky and D. E. Clapham (2004). "The mitochondrial calcium uniporter is a highly selective ion channel." Nature **427**(6972): 360-364.

Kitamura, M. (2011). "Control of NF-kappaB and inflammation by the unfolded protein response." Int Rev Immunol **30**(1): 4-15.

Kiviniemi, H., S. Laitinen, M. Stahlberg, M. Larmi and M. Kairaluoma (1986). "Thyrotropin-releasing hormone in acute pancreatitis. A preliminary report." Acta Chir Scand **152**: 43-47.

Klar, E., T. Foitzik, H. Buhr, K. Messmer and C. Herfarth (1993). "Isovolemic hemodilution with dextran 60 as treatment of pancreatic ischemia in acute pancreatitis. Clinical practicability of an experimental concept." Ann Surg **217**(4): 369-374.

Kobayashi, M., H. Kobayashi, D. N. Herndon, R. B. Pollard and F. Suzuki (1998). "Burn-associated Candida albicans infection caused by CD30+ type 2 T cells." J Leukoc Biol **63**(6): 723-731.

Kowalik, A. S., C. L. Johnson, S. A. Chadi, J. Y. Weston, E. N. Fazio and C. L. Pin (2007). "Mice lacking the transcription factor Mist1 exhibit an altered stress response and increased sensitivity to caerulein-induced pancreatitis." Am J Physiol Gastrointest Liver Physiol **292**(4): G1123-1132.

Kozutsumi, Y., M. Segal, K. Normington, M. J. Gething and J. Sambrook (1988). "The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins." Nature **332**(6163): 462-464.

Krishna, S. G., A. Hinton, V. Oza, P. A. Hart, E. Swei, S. El-Dika, P. P. Stanich, H. Hussan, C. Zhang and D. L. Conwell (2015). "Morbid Obesity Is Associated With Adverse Clinical Outcomes in Acute Pancreatitis: A Propensity-Matched Study." Am J Gastroenterol **110**(11): 1608-1619.

Kronborg, O., S. Bulow, P. M. Joergensen and L. B. Svendsen (1980). "A randomized double-blind trial of glucagon in treatment of first attack of severe acute pancreatitis without associated biliary disease." Am J Gastroenterol **73**(5): 423-425.

Kruger, B., E. Albrecht and M. M. Lerch (2000). "The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis." Am J Pathol **157**(1): 43-50.

Kubisch, C. H. and C. D. Logsdon (2008). "Endoplasmic reticulum stress and the pancreatic acinar cell." Expert Rev Gastroenterol Hepatol **2**(2): 249-260.

Kubisch, C. H., M. D. Sans, T. Arumugam, S. A. Ernst, J. A. Williams and C. D. Logsdon (2006). "Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis." Am J Physiol Gastrointest Liver Physiol **291**(2): G238-245.

Kwanngern, K., P. Tiyapattanaputi, M. Wanitpukdeedecha and P. Navicharern (2005). "Can a single dose corticosteroid reduce the incidence of post-ERCP pancreatitis? A randomized, prospective control study." J Med Assoc Thai **88 Suppl 4**: S42-45.

Kysenius, K., C. A. Brunello and H. J. Huttunen (2014). "Mitochondria and NMDA receptor-dependent toxicity of berberine sensitizes neurons to glutamate and rotenone injury." PLoS One **9**(9): e107129.

Lange, L. G. and B. E. Sobel (1983). "Mitochondrial dysfunction induced by fatty acid ethyl esters, myocardial metabolites of ethanol." J Clin Invest **72**(2): 724-731.

Lankisch, P. G., K. Winckler, M. Bokermann, H. Schmidt and W. Creutzfeldt (1974). "The influence of glucagon on acute experimental pancreatitis in the rat." Scand J Gastroenterol **9**(8): 725-729.

Laposata, E. A. and L. G. Lange (1986). "Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse." Science **231**(4737): 497-499.

Lasztity, N., J. Hamvas, L. Biro, E. Nemeth, T. Marosvolgyi, T. Decsi, A. Pap and M. Antal (2005). "Effect of enterally administered n-3 polyunsaturated fatty acids in acute pancreatitis--a prospective randomized clinical trial." Clin Nutr **24**(2): 198-205.

Laukkarinen, J. M., G. J. Van Acker, E. R. Weiss, M. L. Steer and G. Perides (2007). "A mouse model of acute biliary pancreatitis induced by retrograde pancreatic duct infusion of Na-taurocholate." Gut **56**(11): 1590-1598.

Lavy, A., A. Karban, A. Suissa, K. Yassin, I. Hermesh and A. Ben-Amotz (2004). "Natural beta-carotene for the prevention of post-ERCP pancreatitis." Pancreas **29**(2): e45-50.

Le Lamer, S., S. Paradis, H. Rahmouni, C. Chaimbault, M. Michaud, M. Culcasi, J. Afxantidis, M. Latreille, P. Berna, A. Berdeaux, S. Pietri, D. Morin, Y. Donazzolo, J. L. Abitbol, R. M. Pruss and S. Schaller (2014).

"Translation of TRO40303 from myocardial infarction models to demonstration of safety and tolerance in a randomized Phase I trial." J Transl Med **12**: 38.

Lechin, F., B. Van der Dijs, M. Lechin, H. Jara, A. Lechin, A. Cabrera, I. Rada, B. Orozco, V. Jimenez and T. Valderrama (1992). "Clonidine treatment of acute pancreatitis: report of five cases." Acta Gastroenterol Latinoam **22**(2): 119-124.

Lee, K. T., P. C. Sheen and T. J. Huang (1988). "[The ultrastructural study of acute gallstone pancreatitis]." Gaoxiong Yi Xue Ke Xue Za Zhi **4**(7): 392-400.

Leese, T., M. Holliday, D. Heath, A. W. Hall and P. R. Bell (1987). "Multicentre clinical trial of low volume fresh frozen plasma therapy in acute pancreatitis." Br J Surg **74**(10): 907-911.

Leese, T., M. Holliday, M. Watkins, W. M. Thomas, J. P. Neoptolemos, C. Hall and A. Attard (1991). "A multicentre controlled clinical trial of high-volume fresh frozen plasma therapy in prognostically severe acute pancreatitis." Ann R Coll Surg Engl **73**(4): 207-214.

Lerch, M. M. and F. S. Gorelick (2013). "Models of acute and chronic pancreatitis." Gastroenterology **144**(6): 1180-1193.

Lerch, M. M., W. Halangk and J. Mayerle (2013). "Preventing pancreatitis by protecting the mitochondrial permeability transition pore." Gastroenterology **144**(2): 265-269.

Li, J., J. Chen, C. Gui, L. Zhang, Y. Qin, Q. Xu, J. Zhang, H. Liu, X. Shen and H. Jiang (2006). "Discovering novel chemical inhibitors of human cyclophilin A: virtual screening, synthesis, and bioassay." Bioorg Med Chem **14**(7): 2209-2224.

Li, X., Z. Wu, H. Sha, Z. Wang, Z. Ma, E. Wu and Q. Ma (2015). "Protective effects of polyenoylphosphatidylcholine in rats with severe acute pancreatitis." Pancreas **44**(4): 596-601.

Li, Z. S., X. Pan, W. J. Zhang, B. Gong, F. C. Zhi, X. G. Guo, P. M. Li, Z. N. Fan, W. S. Sun, Y. Z. Shen, S. R. Ma, W. F. Xie, M. H. Chen and Y. Q. Li (2007). "Effect of octreotide administration in the prophylaxis of post-ERCP pancreatitis and hyperamylasemia: A multicenter, placebo-controlled, randomized clinical trial." Am J Gastroenterol **102**(1): 46-51.

Lipinski, C. A., F. Lombardo, B. W. Dominy and P. J. Feeney (2001). "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings." Adv Drug Deliv Rev **46**(1-3): 3-26.

Liu, C., K. Dou, C. Dou, J. Liu and Q. Zhao (2010). "Anti-inflammatory effects of tacrolimus in a rat model of acute pancreatitis." Med Chem **6**(1): 37-43.

Liu, Y., D. Zhou, F. W. Long, K. L. Chen, H. W. Yang, Z. Y. Lv, B. Zhou, Z. H. Peng, X. F. Sun, Y. Li and Z. G. Zhou (2016). "Resolvin D1 protects against inflammation in experimental acute pancreatitis and associated lung injury." Am J Physiol Gastrointest Liver Physiol **310**(5): G303-309.

Loiudice, T. A., J. Lang, H. Mehta and L. Banta (1984). "Treatment of acute alcoholic pancreatitis: the roles of cimetidine and nasogastric suction." Am J Gastroenterol **79**(7): 553-558.

Lombardi, B., L. W. Estes and D. S. Longnecker (1975). "Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by DL-ethionine fed with a choline-deficient diet." Am J Pathol **79**(3): 465-480.

Low, J. T., A. Shukla and P. Thorn (2010). "Pancreatic acinar cell: new insights into the control of secretion." Int J Biochem Cell Biol **42**(10): 1586-1589.

Lu, X. S., F. Qiu, J. Q. Li, Q. Q. Fan, R. G. Zhou, Y. H. Ai, K. C. Zhang and Y. X. Li (2009). "Low molecular weight heparin in the treatment of severe acute pancreatitis: a multiple centre prospective clinical study." Asian J Surg **32**(2): 89-94.

Lur, G., L. P. Haynes, I. A. Prior, O. V. Gerasimenko, S. Feske, O. H. Petersen, R. D. Burgoyne and A. V. Tepikin (2009). "Ribosome-free terminals of rough ER allow formation of STIM1 puncta and segregation of STIM1 from IP(3) receptors." Curr Biol **19**(19): 1648-1653.

Luvisetto, S., E. Basso, V. Petronilli, P. Bernardi and M. Forte (2008). "Enhancement of anxiety, facilitation of avoidance behavior, and occurrence of adult-onset obesity in mice lacking mitochondrial cyclophilin D." Neuroscience **155**(3): 585-596.

Machado, M. C., A. M. Coelho, V. Pontieri, S. N. Sampietre, N. A. Molan, F. Soriano, A. S. Matheus, R. A. Patzina, J. E. Cunha and I. T. Velasco (2006). "Local and systemic effects of hypertonic solution (NaCl 7.5%) in experimental acute pancreatitis." Pancreas **32**(1): 80-86.

Maciejewski, R., F. Burdan, K. Burski, B. Madej, R. Ziemiakowicz, A. Dabrowski and G. Wallner (2005). "Selected biochemical parameters and ultrastructural picture of pancreas due to Ulinastatin treatment of experimental acute pancreatitis." Exp Toxicol Pathol **56**(4-5): 305-311.

Macleod, M. R., T. O'Collins, L. L. Horky, D. W. Howells and G. A. Donnan (2005). "Systematic review and metaanalysis of the efficacy of FK506 in experimental stroke." J Cereb Blood Flow Metab **25**(6): 713-721.

Madern, D. (2002). "Molecular evolution within the L-malate and L-lactate dehydrogenase super-family." J Mol Evol **54**(6): 825-840.

Makhija, R. and A. N. Kingsnorth (2002). "Cytokine storm in acute pancreatitis." J Hepatobiliary Pancreat Surg **9**(4): 401-410.

Maleth, J., A. Balazs, P. Pallagi, Z. Balla, B. Kui, M. Katona, L. Judak, I. Nemeth, L. V. Kemeny, Z. Rakonczay, Jr., V. Venglovecz, I. Foldesi, Z. Peto, A. Somoracz, K. Borka, D. Perdomo, G. L. Lukacs, M. A. Gray, S. Monterisi, M. Zaccolo, M. Sendler, J. Mayerle, J. P. Kuhn, M. M. Lerch, M. Sahin-Toth and P. Hegyi (2015). "Alcohol disrupts levels and function of the cystic fibrosis transmembrane conductance regulator to promote development of pancreatitis." Gastroenterology **148**(2): 427-439 e416.

Maleth, J. and P. Hegyi (2016). "Ca2+ toxicity and mitochondrial damage in acute pancreatitis: translational overview." Philos Trans R Soc Lond B Biol Sci **371**(1700).

Maleth, J., Z. Rakonczay, Jr., V. Venglovecz, N. J. Dolman and P. Hegyi (2013). "Central role of mitochondrial injury in the pathogenesis of acute pancreatitis." Acta Physiol (Oxf) **207**(2): 226-235.

Manes, G., S. Ardizzone, G. Lombardi, G. Uomo, O. Pieramico and G. B. Porro (2007). "Efficacy of postprocedure administration of gabexate mesylate in the prevention of post-ERCP pancreatitis: a randomized, controlled, multicenter study." Gastrointest Endosc **65**(7): 982-987.

Manolakopoulos, S., A. Avgerinos, J. Vlachogiannakos, A. Armonis, N. Viazis, N. Papadimitriou, N. Mathou, G. Stefanidis, G. Rekoumis, E. Vienna, D. Tzourmakliotis and S. A. Raptis (2002). "Octreotide versus hydrocortisone versus placebo in the prevention of post-ERCP pancreatitis: a multicenter randomized controlled trial." Gastrointest Endosc **55**(4): 470-475.

Marks, A. R. (1996). "Cellular functions of immunophilins." Physiol Rev **76**(3): 631-649.

Martinez-Torres, H., X. Rodriguez-Lomeli, C. Davalos-Cobian, J. Garcia-Correa, J. M. Maldonado-Martinez, F. Medrano-Munoz, C. Fuentes-Orozco and A. Gonzalez-Ojeda (2009). "Oral allopurinol to prevent hyperamylasemia and acute pancreatitis after endoscopic retrograde cholangiopancreatography." World J Gastroenterol **15**(13): 1600-1606.

Martinez, E. and F. Navarrete (1984). "A controlled trial of synthetic salmon calcitonin in the treatment of severe acute pancreatitis." World J Surg **8**(3): 354-359.

Masamune, A. (2014). "Genetics of pancreatitis: the 2014 update." Tohoku J Exp Med **232**(2): 69-77.

Mason, J. and A. K. Siriwardena (2005). "Designing future clinical trials in acute pancreatitis." Pancreatology **5**(2-3): 113-115.

Matheus, A. S., A. M. Coelho, S. Sampietre, J. Jukemura, R. A. Patzina, J. E. Cunha and M. C. Machado (2009). "Do the effects of pentoxifylline on the inflammatory process and pancreatic infection justify its use in acute pancreatitis?" Pancreatology **9**(5): 687-693.

Mayer, J. M., V. J. Laine, A. Gezgin, S. Kolodziej, T. J. Nevalainen, M. Storck and H. G. Beger (2000). "Single doses of FK506 and OKT3 reduce severity in early experimental acute pancreatitis." Eur J Surg **166**(9): 734-741.

McKay, C. J., F. Curran, C. Sharples, J. N. Baxter and C. W. Imrie (1997). "Prospective placebo-controlled randomized trial of lexipafant in predicted severe acute pancreatitis." Br J Surg **84**(9): 1239-1243.

Milewski, J., G. Rydzewska, M. Degowska, M. Kierzkiewicz and A. Rydzewski (2006). "N-acetylcysteine does not prevent post-endoscopic

retrograde cholangiopancreatography hyperamylasemia and acute pancreatitis." World J Gastroenterol **12**(23): 3751-3755.

Miranda, C. J., J. M. Mason, B. I. Babu, A. J. Sheen, J. M. Eddleston, M. J. Parker, P. Pemberton and A. K. Siriwardena (2015). "Twenty-four hour infusion of human recombinant activated protein C (Xigris) early in severe acute pancreatitis: The XIG-AP 1 trial." Pancreatology **15**(6): 635-641.

Mittal, A., A. J. Hickey, C. C. Chai, B. P. Loveday, N. Thompson, A. Dare, B. Delahunt, G. J. Cooper, J. A. Windsor and A. R. Phillips (2011). "Early organ-specific mitochondrial dysfunction of jejunum and lung found in rats with experimental acute pancreatitis." HPB (Oxford) **13**(5): 332-341.

Mizunuma, T., S. Kawamura and Y. Kishino (1984). "Effects of injecting excess arginine on rat pancreas." J Nutr **114**(3): 467-471.

Mooren, F., V. Hlouschek, T. Finkes, S. Turi, I. A. Weber, J. Singh, W. Domschke, J. Schnekenburger, B. Kruger and M. M. Lerch (2003). "Early changes in pancreatic acinar cell calcium signaling after pancreatic duct obstruction." J Biol Chem **278**(11): 9361-9369.

Moreno-Otero, R., S. Rodriguez, J. Carbo, L. Garcia-Buey and J. M. Pajares (1989). "Double-blind trial of pirenzepine in acute pancreatitis." Digestion **42**(1): 51-56.

Morimoto, R. I. (1993). "Cells in stress: transcriptional activation of heat shock genes." Science **259**(5100): 1409-1410.

Morin, D., J. Musman, S. Pons, A. Berdeaux and B. Ghaleh (2016). "Mitochondrial translocator protein (TSPO): From physiology to cardioprotection." Biochem Pharmacol **105**: 1-13.

Moriwaki, K. and F. K. Chan (2013). "RIP3: a molecular switch for necrosis and inflammation." Genes Dev **27**(15): 1640-1649.

Moynihan, B. (1925). "Acute Pancreatitis." Ann Surg **81**(1): 132-142.

Muik, M., R. Schindl, M. Fahrner and C. Romanin (2012). "Ca(2+) release-activated Ca(2+) (CRAC) current, structure, and function." Cell Mol Life Sci **69**(24): 4163-4176.

Mukherjee, R., O. A. Mareninova, I. V. Odinokova, W. Huang, J. Murphy, M. Chvanov, M. A. Javed, L. Wen, D. M. Booth, M. C. Cane, M. Awais, B. Gavillet, R. M. Pruss, S. Schaller, J. D. Molkentin, A. V. Tepikin, O. H. Petersen, S. J. Pandol, I. Gukovsky, D. N. Criddle, A. S. Gukovskaya, R. Sutton and N. P. B. R. U. and (2015). "Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP." Gut.

Mumcu, S., E. Alhan, S. Turkyilmaz, B. V. Kural, C. Ercin and N. I. Kalyoncu (2005). "Effects of N-acetylcysteine on acute necrotizing pancreatitis in rats." Eur Surg Res **37**(3): 173-178.

Murphy, J. A., D. N. Criddle, M. Sherwood, M. Chvanov, R. Mukherjee, E. McLaughlin, D. Booth, J. V. Gerasimenko, M. G. Raraty, P. Ghaneh, J. P. Neoptolemos, O. V. Gerasimenko, A. V. Tepikin, G. M. Green, J. R. Reeve, Jr., O. H. Petersen and R. Sutton (2008). "Direct activation of cytosolic Ca2+ signaling and enzyme secretion by cholecystokinin in human pancreatic acinar cells." Gastroenterology **135**(2): 632-641.

Nakagawa, T., S. Shimizu, T. Watanabe, O. Yamaguchi, K. Otsu, H. Yamagata, H. Inohara, T. Kubo and Y. Tsujimoto (2005). "Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death." Nature **434**(7033): 652-658.

Navarro, S., E. Ros, R. Aused, M. Garcia Puges, J. M. Pique and J. Vilar Bonet (1984). "Comparison of fasting, nasogastric suction and cimetidine in the treatment of acute pancreatitis." Digestion **30**(4): 224-230.

Nevalainen, T. J. and A. Seppa (1975). "Acute pancreatitis caused by closed duodenal loop in the rat." Scand J Gastroenterol **10**(5): 521-527.

Ni, S., Y. Yuan, J. Huang, X. Mao, M. Lv, J. Zhu, X. Shen, J. Pei, L. Lai, H. Jiang and J. Li (2009). "Discovering potent small molecule inhibitors of cyclophilin A using de novo drug design approach." J Med Chem **52**(17): 5295-5298.

Niederau, C., L. D. Ferrell and J. H. Grendell (1985). "Caerulein-induced acute necrotizing pancreatitis in mice: protective effects of proglumide, benzotript, and secretin." Gastroenterology **88**(5 Pt 1): 1192-1204.

Nitsche, C., S. Maertin, J. Scheiber, C. A. Ritter, M. M. Lerch and J. Mayerle (2012). "Drug-induced pancreatitis." Curr Gastroenterol Rep **14**(2): 131-138.

Norman, J. (1998). "The role of cytokines in the pathogenesis of acute pancreatitis." Am J Surg **175**(1): 76-83.

Nuhn, P., T. Mitkus, G. O. Ceyhan, B. M. Kunzli, F. Bergmann, L. Fischer, N. Giese, H. Friess and P. O. Berberat (2013). "Heme oxygenase 1-generated carbon monoxide and biliverdin attenuate the course of experimental necrotizing pancreatitis." Pancreas **42**(2): 265-271.

Ochi, K., H. Harada and K. Satake (1999). "Clinical evaluation of cholecystokinin-A- receptor antagonist (loxiglumide) for the treatment of acute pancreatitis. A preliminary clinical trial. Study Group of Loxiglumide in Japan." Digestion **60 Suppl 1**: 81-85.

Oiva, J., H. Mustonen, M. L. Kylanpaa, K. Kuuliala, S. Siitonen, E. Kemppainen, P. Puolakkainen and H. Repo (2013). "Patients with acute pancreatitis complicated by organ dysfunction show abnormal peripheral blood polymorphonuclear leukocyte signaling." Pancreatology **13**(2): 118-124.

Oiva, J., H. Mustonen, M. L. Kylanpaa, L. Kyhala, T. Alanara, S. Aittomaki, S. Siitonen, E. Kemppainen, P. Puolakkainen and H. Repo (2010). "Patients with acute pancreatitis complicated by organ failure show highly aberrant monocyte signaling profiles assessed by phospho-specific flow cytometry." Crit Care Med **38**(8): 1702-1708.

Orabi, A. I., K. A. Muili, D. Wang, S. Jin, G. Perides and S. Z. Husain (2013). "Preparation of pancreatic acinar cells for the purpose of calcium imaging, cell injury measurements, and adenoviral infection." J Vis Exp(77): e50391.

Orrenius, S., B. Zhivotovsky and P. Nicotera (2003). "Regulation of cell death: the calcium-apoptosis link." Nat Rev Mol Cell Biol **4**(7): 552-565.

Osman, M. O., S. B. Lausten, N. O. Jakobsen, J. U. Kristensen, B. Deleuran, C. G. Larsen and S. L. Jensen (1999). "Graded experimental acute pancreatitis: monitoring of a renewed rabbit model focusing on the production of interleukin-8 (IL-8) and CD11b/CD18." Eur J Gastroenterol Hepatol **11**(2): 137-149.

Otsuka, T., S. Kawazoe, S. Nakashita, S. Kamachi, S. Oeda, C. Sumida, T. Akiyama, K. Ario, M. Fujimoto, M. Tabuchi and T. Noda (2012). "Low-dose rectal diclofenac for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis: a randomized controlled trial." J Gastroenterol **47**(8): 912-917.

pancreadpedia. (2017). "Classic Books and Papers in Pancreatology." Retrieved 8 October, 2017, from https://www.pancreapedia.org/classic-books-and-papers-in-pancreatology.

Pandol, S. J., A. K. Saluja, C. W. Imrie and P. A. Banks (2007). "Acute pancreatitis: bench to the bedside." Gastroenterology **132**(3): 1127-1151.

Papadopoulos, V., H. Amri, N. Boujrad, C. Cascio, M. Culty, M. Garnier, M. Hardwick, H. Li, B. Vidic, A. S. Brown, J. L. Reversa, J. M. Bernassau and K. Drieu (1997). "Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis." Steroids **62**(1): 21-28.

Paradis, S., V. Leoni, C. Caccia, A. Berdeaux and D. Morin (2013). "Cardioprotection by the TSPO ligand 4'-chlorodiazepam is associated with inhibition of mitochondrial accumulation of cholesterol at reperfusion." Cardiovasc Res **98**(3): 420-427.

Parekh, A. B. and J. W. Putney, Jr. (2005). "Store-operated calcium channels." Physiol Rev **85**(2): 757-810.

Park, J. Y., T. J. Jeon, M. W. Hwang, D. H. Sinn, T. H. Oh, W. C. Shin and W. C. Choi (2014). "Comparison between ulinastatin and nafamostat for prevention of post-endoscopic retrograde cholangiopancreatography complications: a prospective, randomized trial." Pancreatology **14**(4): 263-267.

Park, M. K., M. C. Ashby, G. Erdemli, O. H. Petersen and A. V. Tepikin (2001). "Perinuclear, perigranular and sub-plasmalemmal mitochondria have distinct functions in the regulation of cellular calcium transport." EMBO J **20**(8): 1863-1874.

Park, S. W., M. J. Chung, T. G. Oh, J. Y. Park, S. Bang, S. W. Park and S. Y. Song (2015). "Intramuscular diclofenac for the prevention of post-ERCP pancreatitis: a randomized trial." Endoscopy **47**(1): 33-39.

Paszkowski, A. S., B. Rau, J. M. Mayer, P. Moller and H. G. Beger (2002). "Therapeutic application of caspase 1/interleukin-1beta-converting enzyme inhibitor decreases the death rate in severe acute experimental pancreatitis." Ann Surg **235**(1): 68-76.

Paterniti, I., E. Mazzon, L. Riccardi, M. Galuppo, D. Impellizzeri, E. Esposito, P. Bramanti, A. Cappellani and S. Cuzzocrea (2012). "Peroxisome

proliferator-activated receptor beta/delta agonist GW0742 ameliorates cerulein- and taurocholate-induced acute pancreatitis in mice." Surgery **152**(1): 90-106.

Pederzoli, P., G. Cavallini, M. Falconi and C. Bassi (1993). "Gabexate mesilate vs aprotinin in human acute pancreatitis (GA.ME.P.A.). A prospective, randomized, double-blind multicenter study." Int J Pancreatol **14**(2): 117-124.

Peery, A. F., S. D. Crockett, A. S. Barritt, E. S. Dellon, S. Eluri, L. M. Gangarosa, E. T. Jensen, J. L. Lund, S. Pasricha, T. Runge, M. Schmidt, N. J. Shaheen and R. S. Sandler (2015). "Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States." Gastroenterology **149**(7): 1731-1741 e1733.

Petersen, O. H. (2005). "Ca2+ signalling and Ca2+-activated ion channels in exocrine acinar cells." Cell Calcium **38**(3-4): 171-200.

Petersen, O. H. and R. Sutton (2006). "Ca2+ signalling and pancreatitis: effects of alcohol, bile and coffee." Trends Pharmacol Sci **27**(2): 113-120.

Petersen, O. H. and A. V. Tepikin (2008). "Polarized calcium signaling in exocrine gland cells." Annu Rev Physiol **70**: 273-299.

Petrov, M. S., S. Shanbhag, M. Chakraborty, A. R. Phillips and J. A. Windsor (2010). "Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis." Gastroenterology **139**(3): 813-820.

Pettila, V., L. Kyhala, M. L. Kylanpaa, A. Leppaniemi, M. Tallgren, A. Markkola, P. Puolakkainen, H. Repo and E. Kemppainen (2010). "APCAP--activated protein C in acute pancreatitis: a double-blind randomized human pilot trial." Crit Care **14**(4): R139.

Pezzilli, R. (2009). "Pharmacotherapy for acute pancreatitis." Expert Opin Pharmacother **10**(18): 2999-3014.

Pezzilli, R., G. Uomo, A. Gabbrielli, A. Zerbi, L. Frulloni, P. De Rai, L. Castoldi, G. Cavallini, V. Di Carlo and A. S. G. ProInf (2007). "A prospective multicentre survey on the treatment of acute pancreatitis in Italy." Dig Liver Dis **39**(9): 838-846.

Phillip, V., W. Huber, F. Hagemes, S. Lorenz, U. Matheis, S. Preinfalk, T. Schuster, F. Lippl, B. Saugel and R. M. Schmid (2011). "Incidence of acute pancreatitis does not increase during Oktoberfest, but is higher than previously described in Germany." Clin Gastroenterol Hepatol **9**(11): 995-1000 e1003.

Piascik, M., G. Rydzewska, J. Milewski, S. Olszewski, M. Furmanek, J. Walecki and A. Gabryelewicz (2010). "The results of severe acute pancreatitis treatment with continuous regional arterial infusion of protease inhibitor and antibiotic: a randomized controlled study." Pancreas **39**(6): 863-867.

Piot, C., P. Croisille, P. Staat, H. Thibault, G. Rioufol, N. Mewton, R. Elbelghiti, T. T. Cung, E. Bonnefoy, D. Angoulvant, C. Macia, F. Raczka, C. Sportouch, G. Gahide, G. Finet, X. Andre-Fouet, D. Revel, G. Kirkorian, J. P. Monassier, G. Derumeaux and M. Ovize (2008). "Effect of cyclosporine on reperfusion injury in acute myocardial infarction." N Engl J Med **359**(5): 473-481.

Polla, B. S., M. Perin and L. Pizurki (1993). "Regulation and functions of stress proteins in allergy and inflammation." Clin Exp Allergy **23**(7): 548-556.

Prat, F., J. Amaris, B. Ducot, M. Bocquentin, J. Fritsch, A. D. Choury, G. Pelletier and C. Buffet (2002). "Nifedipine for prevention of post-ERCP

pancreatitis: a prospective, double-blind randomized study." Gastrointest Endosc **56**(2): 202-208.

Rabenstein, T., B. Fischer, V. Wiessner, H. Schmidt, M. Radespiel-Troger, J. Hochberger, S. Muhldorfer, G. Nusko, H. Messmann, J. Scholmerich, H. J. Schulz, H. Schonekas, E. G. Hahn and H. T. Schneider (2004). "Low-molecular-weight heparin does not prevent acute post-ERCP pancreatitis." Gastrointest Endosc **59**(6): 606-613.

Rakonczay, Z., Jr., P. Hegyi, T. Takacs, J. McCarroll and A. K. Saluja (2008). "The role of NF-kappaB activation in the pathogenesis of acute pancreatitis." Gut **57**(2): 259-267.

Raraty, M., J. Ward, G. Erdemli, C. Vaillant, J. P. Neoptolemos, R. Sutton and O. H. Petersen (2000). "Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells." Proc Natl Acad Sci U S A **97**(24): 13126-13131.

Rasola, A. and P. Bernardi (2011). "Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis." Cell Calcium **50**(3): 222-233.

Ray, K. (2015). "Pancreatitis: MPTP pore opening proves crucial for experimental pancreatitis." Nat Rev Gastroenterol Hepatol **12**(8): 428.

Razavi, D., M. Lindblad, T. Bexelius, V. Oskarsson, O. Sadr-Azodi and R. Ljung (2016). "Polypharmacy and risk of acute pancreatitis." Pharmacoepidemiol Drug Saf **25**(11): 1337-1341.

Regan, P. T., J. R. Malagelada, V. L. Go, A. M. Wolf and E. P. DiMagno (1981). "A prospective study of the antisecretory and therapeutic effects of cimetidine and glucagon in human acute pancreatitis." Mayo Clin Proc **56**(8): 499-503.

Ren, J., Z. Luo, F. Tian, Q. Wang, K. Li and C. Wang (2012). "Hydrogen-rich saline reduces the oxidative stress and relieves the severity of trauma-induced acute pancreatitis in rats." J Trauma Acute Care Surg **72**(6): 1555-1561.

Reutenauer, J., O. M. Dorchies, O. Patthey-Vuadens, G. Vuagniaux and U. T. Ruegg (2008). "Investigation of Debio 025, a cyclophilin inhibitor, in the dystrophic mdx mouse, a model for Duchenne muscular dystrophy." Br J Pharmacol **155**(4): 574-584.

Richter, F., F. Gao, V. Medvedeva, P. Lee, N. Bove, S. M. Fleming, M. Michaud, V. Lemesre, S. Patassini, K. De La Rosa, C. K. Mulligan, P. C. Sioshansi, C. Zhu, G. Coppola, T. Bordet, R. M. Pruss and M. F. Chesselet (2014). "Chronic administration of cholesterol oximes in mice increases transcription of cytoprotective genes and improves transcriptome alterations induced by alpha-synuclein overexpression in nigrostriatal dopaminergic neurons." Neurobiol Dis **69**: 263-275.

Rizzuto, R. and T. Pozzan (2006). "Microdomains of intracellular Ca2+: molecular determinants and functional consequences." Physiol Rev **86**(1): 369-408.

Roberts, S. E., S. Morrison-Rees, A. John, J. G. Williams, T. H. Brown and D. G. Samuel (2017). "The incidence and aetiology of acute pancreatitis across Europe." Pancreatology **17**(2): 155-165.

Robertson, C. S., G. S. Basran and J. G. Hardy (1988). "Lung vascular permeability in patients with acute pancreatitis." Pancreas **3**(2): 162-165.

Romagnuolo, J., R. Hilsden, G. S. Sandha, M. Cole, S. Bass, G. May, J. Love, V. G. Bain, J. McKaigney and R. N. Fedorak (2008). "Allopurinol to prevent pancreatitis after endoscopic retrograde cholangiopancreatography:

a randomized placebo-controlled trial." Clin Gastroenterol Hepatol **6**(4): 465-471; quiz 371.

Ron, D. and P. Walter (2007). "Signal integration in the endoplasmic reticulum unfolded protein response." Nat Rev Mol Cell Biol **8**(7): 519-529.

Rongione, A. J., A. M. Kusske, K. Kwan, S. W. Ashley, H. A. Reber and D. W. McFadden (1997). "Interleukin 10 reduces the severity of acute pancreatitis in rats." Gastroenterology **112**(3): 960-967.

Rupprecht, R., V. Papadopoulos, G. Rammes, T. C. Baghai, J. Fan, N. Akula, G. Groyer, D. Adams and M. Schumacher (2010). "Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders." Nat Rev Drug Discov **9**(12): 971-988.

Saenko, V. F., V. I. Lupal'tsov, G. F. Babenkov, V. M. Kopchak and A. P. Volzhanskii (1999). "[The administration of sandostatin in the combined treatment of acute pancreatitis and its complications]." Klin Khir(10): 5-7.

Sah, R. P., P. Garg and A. K. Saluja (2012). "Pathogenic mechanisms of acute pancreatitis." Curr Opin Gastroenterol **28**(5): 507-515.

Sah, R. P., S. K. Garg, A. K. Dixit, V. Dudeja, R. K. Dawra and A. K. Saluja (2014). "Endoplasmic reticulum stress is chronically activated in chronic pancreatitis." J Biol Chem **289**(40): 27551-27561.

Sakamuru, S., M. S. Attene-Ramos and M. Xia (2016). "Mitochondrial Membrane Potential Assay." Methods Mol Biol **1473**: 17-22.

Saluja, A., S. Hashimoto, M. Saluja, R. E. Powers, J. Meldolesi and M. L. Steer (1987). "Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis." Am J Physiol **253**(4 Pt 1): G508-516.

Saluja, A. K., L. Bhagat, H. S. Lee, M. Bhatia, J. L. Frossard and M. L. Steer (1999). "Secretagogue-induced digestive enzyme activation and cell injury in rat pancreatic acini." Am J Physiol **276**(4 Pt 1): G835-842.

Samad, A., A. James, J. Wong, P. Mankad, J. Whitehouse, W. Patel, M. Alves-Simoes, A. K. Siriwardena and J. I. Bruce (2014). "Insulin protects pancreatic acinar cells from palmitoleic acid-induced cellular injury." J Biol Chem **289**(34): 23582-23595.

Schaller, S., M. Michaud, V. Latyszenok, F. Robert, M. Hocine, T. Arnoux, M. Gabriac, H. Codoul, A. Bourhane, I. C. de Bellefois, J. Afxantidis and R. M. Pruss (2015). "TRO40303, a mitochondrial-targeted cytoprotective compound, provides protection in hepatitis models." Pharmacol Res Perspect **3**(3): e00144.

Schaller, S., S. Paradis, G. A. Ngoh, R. Assaly, B. Buisson, C. Drouot, M. A. Ostuni, J. J. Lacapere, F. Bassissi, T. Bordet, A. Berdeaux, S. P. Jones, D. Morin and R. M. Pruss (2010). "TRO40303, a new cardioprotective compound, inhibits mitochondrial permeability transition." J Pharmacol Exp Ther **333**(3): 696-706.

Schild, L., R. Matthias, A. Stanarius, G. Wolf, W. Augustin and W. Halangk (1999). "Induction of permeability transition in pancreatic mitochondria by cerulein in rats." Mol Cell Biochem **195**(1-2): 191-197.

Schinzel, A. C., O. Takeuchi, Z. Huang, J. K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N. N. Danial, M. A. Moskowitz and S. J. Korsmeyer (2005). "Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia." Proc Natl Acad Sci U S A **102**(34): 12005-12010.

Schmidt, J., D. W. Rattner, K. Lewandrowski, C. C. Compton, U. Mandavilli, W. T. Knoefel and A. L. Warshaw (1992). "A better model of acute pancreatitis for evaluating therapy." Ann Surg **215**(1): 44-56.

Schulz, K. F., D. G. Altman and D. Moher (2010). "CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials." J Pharmacol Pharmacother **1**(2): 100-107.

Sena, E., H. B. van der Worp, D. Howells and M. Macleod (2007). "How can we improve the pre-clinical development of drugs for stroke?" Trends Neurosci **30**(9): 433-439.

Sendler, M., A. Dummer, F. U. Weiss, B. Kruger, T. Wartmann, K. Scharffetter-Kochanek, N. van Rooijen, S. R. Malla, A. Aghdassi, W. Halangk, M. M. Lerch and J. Mayerle (2013). "Tumour necrosis factor alpha secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice." Gut **62**(3): 430-439.

Seyhun, E., A. Malo, C. Schafer, C. A. Moskaluk, R. T. Hoffmann, B. Goke and C. H. Kubisch (2011). "Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, acinar cell damage, and systemic inflammation in acute pancreatitis." Am J Physiol Gastrointest Liver Physiol **301**(5): G773-782.

Shah, T. U., R. Liddle, M. S. Branch, P. Jowell, J. Obando and M. Poleski (2012). "Pilot study of aprepitant for prevention of post-ERCP pancreatitis in high risk patients: a phase II randomized, double-blind placebo controlled trial." JOP **13**(5): 514-518.

Shalbueva, N., O. A. Mareninova, A. Gerloff, J. Yuan, R. T. Waldron, S. J. Pandol and A. S. Gukovskaya (2013). "Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis." Gastroenterology **144**(2): 437-446 e436.

Sherman, S., W. M. Alazmi, G. A. Lehman, J. E. Geenen, R. Chuttani, R. A. Kozarek, W. D. Welch, S. Souza, J. Pribble and P. A. F. A. H. E. S. G. r (2009). "Evaluation of recombinant platelet-activating factor

acetylhydrolase for reducing the incidence and severity of post-ERCP acute pancreatitis." Gastrointest Endosc **69**(3 Pt 1): 462-472.

Shore, E. R., M. Awais, N. M. Kershaw, R. R. Gibson, S. Pandalaneni, D. Latawiec, L. Wen, M. A. Javed, D. N. Criddle, N. Berry, P. M. O'Neill, L. Y. Lian and R. Sutton (2016). "Small Molecule Inhibitors of Cyclophilin D To Protect Mitochondrial Function as a Potential Treatment for Acute Pancreatitis." J Med Chem **59**(6): 2596-2611.

Sileikyte, J., E. Blachly-Dyson, R. Sewell, A. Carpi, R. Menabo, F. Di Lisa, F. Ricchelli, P. Bernardi and M. Forte (2014). "Regulation of the mitochondrial permeability transition pore by the outer membrane does not involve the peripheral benzodiazepine receptor (Translocator Protein of 18 kDa (TSPO))." J Biol Chem **289**(20): 13769-13781.

Sileikyte, J. and M. Forte (2016). "Shutting down the pore: The search for small molecule inhibitors of the mitochondrial permeability transition." Biochim Biophys Acta **1857**(8): 1197-1202.

Siriwardena, A. K., J. M. Mason, S. Balachandra, A. Bagul, S. Galloway, L. Formela, J. G. Hardman and S. Jamdar (2007). "Randomised, double blind, placebo controlled trial of intravenous antioxidant (n-acetylcysteine, selenium, vitamin C) therapy in severe acute pancreatitis." Gut **56**(10): 1439-1444.

Siriwardena, A. K. and D. A. O'Reilly (2017). "Improving care for patients with pancreatitis." Br J Surg.

Stelzer, A. C., R. W. Frazee, C. Van Huis, J. Cleary, A. W. Opipari, Jr., G. D. Glick and H. M. Al-Hashimi (2010). "NMR studies of an immunomodulatory benzodiazepine binding to its molecular target on the mitochondrial F(1)F(0)-ATPase." Biopolymers **93**(1): 85-92.

Stroke Therapy Academic Industry, R. (1999). "Recommendations for standards regarding preclinical neuroprotective and restorative drug development." Stroke **30**(12): 2752-2758.

Svensson, J. O. (1975). "Role of intravenously infused insulin in treatment of acute pancreatitis. A double-blind study." Scand J Gastroenterol **10**(5): 487-490.

Szatmary, P. (2016). Pancreatitis. J. A. Williams. U.S.A, Michigan Publishing.

Szentesi, A., E. Toth, E. Balint, J. Fanczal, T. Madacsy, D. Laczko, I. Ignath, A. Balazs, P. Pallagi, J. Maleth, Z. Rakonczay, Jr., B. Kui, D. Illes, K. Marta, A. Blasko, A. Demcsak, A. Parniczky, G. Par, S. Godi, D. Mosztbacher, A. Szucs, A. Halasz, F. Izbeki, N. Farkas, P. Hegyi and G. Hungarian Pancreatic Study (2016). "Analysis of Research Activity in Gastroenterology: Pancreatitis Is in Real Danger." PLoS One **11**(10): e0165244.

Takahashi, H., Y. Tsuda, M. Kobayashi, D. N. Herndon and F. Suzuki (2006). "CCL2 as a trigger of manifestations of compensatory anti-inflammatory response syndrome in mice with severe systemic inflammatory response syndrome." J Leukoc Biol **79**(4): 789-796.

Taketani, S., H. Kohno, M. Okuda, T. Furukawa and R. Tokunaga (1994). "Induction of peripheral-type benzodiazepine receptors during differentiation of mouse erythroleukemia cells. A possible involvement of these receptors in heme biosynthesis." J Biol Chem **269**(10): 7527-7531.

Talukdar, R., A. Sareen, H. Zhu, Z. Yuan, A. Dixit, H. Cheema, J. George, U. Barlass, R. Sah, S. K. Garg, S. Banerjee, P. Garg, V. Dudeja, R. Dawra and A. K. Saluja (2016). "Release of Cathepsin B in Cytosol Causes Cell Death in Acute Pancreatitis." Gastroenterology **151**(4): 747-758 e745.

Tashiro, M., C. Schafer, H. Yao, S. A. Ernst and J. A. Williams (2001). "Arginine induced acute pancreatitis alters the actin cytoskeleton and increases heat shock protein expression in rat pancreatic acinar cells." Gut **49**(2): 241-250.

Teich, N., H. Bodeker and V. Keim (2002). "Cathepsin B cleavage of the trypsinogen activation peptide." BMC Gastroenterol **2**: 16.

Thrower, E. C., F. S. Gorelick and S. Z. Husain (2010). "Molecular and cellular mechanisms of pancreatic injury." Curr Opin Gastroenterol **26**(5): 484-489.

Tinel, H., J. M. Cancela, H. Mogami, J. V. Gerasimenko, O. V. Gerasimenko, A. V. Tepikin and O. H. Petersen (1999). "Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca(2+) signals." EMBO J **18**(18): 4999-5008.

Tissieres, A., H. K. Mitchell and U. M. Tracy (1974). "Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs." J Mol Biol **84**(3): 389-398.

Tonetti, F. and A. Calvi (1989). "[5-Fluorouracil in the treatment of acute pancreatitis. Our experience]." Minerva Dietol Gastroenterol **35**(3): 201-204.

Turkyilmaz, S., E. Alhan, C. Ercin, B. Kural Vanizor, N. Kaklikkaya, B. Ates, S. Erdogan and S. Topaloglu (2008). "Effects of caffeic acid phenethyl ester on pancreatitis in rats." J Surg Res **145**(1): 19-24.

Tykka, H. T., E. J. Vaittinen, K. L. Mahlberg, J. E. Railo, P. J. Pantzar, S. Sarna and T. Tallberg (1985). "A randomized double-blind study using CaNa2EDTA, a phospholipase A2 inhibitor, in the management of human acute pancreatitis." Scand J Gastroenterol **20**(1): 5-12.

Uysal, B., M. Yasar, N. Ersoz, O. Coskun, A. Kilic, T. Cayc, B. Kurt, S. Oter, A. Korkmaz and A. Guven (2010). "Efficacy of hyperbaric oxygen therapy and medical ozone therapy in experimental acute necrotizing pancreatitis." Pancreas **39**(1): 9-15.

Valasani, K. R., J. R. Vangavaragu, V. W. Day and S. S. Yan (2014). "Structure based design, synthesis, pharmacophore modeling, virtual screening, and molecular docking studies for identification of novel cyclophilin D inhibitors." J Chem Inf Model **54**(3): 902-912.

Van Acker, G. J., A. K. Saluja, L. Bhagat, V. P. Singh, A. M. Song and M. L. Steer (2002). "Cathepsin B inhibition prevents trypsinogen activation and reduces pancreatitis severity." Am J Physiol Gastrointest Liver Physiol **283**(3): G794-800.

Van Acker, G. J., E. Weiss, M. L. Steer and G. Perides (2007). "Cause-effect relationships between zymogen activation and other early events in secretagogue-induced acute pancreatitis." Am J Physiol Gastrointest Liver Physiol **292**(6): G1738-1746.

van Brunschot, S., R. A. Hollemans, O. J. Bakker, M. G. Besselink, T. H. Baron, H. G. Beger, M. A. Boermeester, T. L. Bollen, M. J. Bruno, R. Carter, J. J. French, D. Coelho, B. Dahl, M. G. Dijkgraaf, N. Doctor, P. J. Fagenholz, G. Farkas, C. F. D. Castillo, P. Fockens, M. L. Freeman, T. B. Gardner, H. V. Goor, H. G. Gooszen, G. Hannink, R. Lochan, C. J. McKay, J. P. Neoptolemos, A. Olah, R. W. Parks, M. P. Peev, M. Raraty, B. Rau, T. Rosch, M. Rovers, H. Seifert, A. K. Siriwardena, K. D. Horvath and H. C. van Santvoort (2017). "Minimally invasive and endoscopic versus open necrosectomy for necrotising pancreatitis: a pooled analysis of individual data for 1980 patients." Gut.

Van Buren, C. T., S. M. Flechner, R. H. Kerman, W. Vaughn and B. D. Kahan (1984). "Cyclosporine improves outcome in high-risk cadaveric renal allograft recipients." Transplant Proc **16**(5): 1162-1166.

van Dijk, S. M., N. D. L. Hallensleben, H. C. van Santvoort, P. Fockens, H. van Goor, M. J. Bruno, M. G. Besselink and G. Dutch Pancreatitis Study (2017). "Acute pancreatitis: recent advances through randomised trials." Gut **66**(11): 2024-2032.

van Grinsven, J., H. C. van Santvoort, M. A. Boermeester, C. H. Dejong, C. H. van Eijck, P. Fockens, M. G. Besselink and G. Dutch Pancreatitis Study (2016). "Timing of catheter drainage in infected necrotizing pancreatitis." Nat Rev Gastroenterol Hepatol **13**(5): 306-312.

Vandenabeele, P., L. Galluzzi, T. Vanden Berghe and G. Kroemer (2010). "Molecular mechanisms of necroptosis: an ordered cellular explosion." Nat Rev Mol Cell Biol **11**(10): 700-714.

Vaquero, E., X. Molero, X. Tian, A. Salas and J. R. Malagelada (1999). "Myofibroblast proliferation, fibrosis, and defective pancreatic repair induced by cyclosporin in rats." Gut **45**(2): 269-277.

Vege, S. S., T. Atwal, Y. Bi, S. T. Chari, M. A. Clemens and F. T. Enders (2015). "Pentoxifylline Treatment in Severe Acute Pancreatitis: A Pilot, Double-Blind, Placebo-Controlled, Randomized Trial." Gastroenterology **149**(2): 318-320 e313.

Vidavalur, R., S. Swarnakar, M. Thirunavukkarasu, S. M. Samuel and N. Maulik (2008). "Ex vivo and in vivo approaches to study mechanisms of cardioprotection targeting ischemia/reperfusion (i/r) injury: useful techniques for cardiovascular drug discovery." Curr Drug Discov Technol **5**(4): 269-278.

Virlos, I. T., J. Mason, D. Schofield, R. F. McCloy, J. M. Eddleston and A. K. Siriwardena (2003). "Intravenous n-acetylcysteine, ascorbic acid and

selenium-based anti-oxidant therapy in severe acute pancreatitis." Scand J Gastroenterol **38**(12): 1262-1267.

Volzke, H., S. E. Baumeister, D. Alte, W. Hoffmann, C. Schwahn, P. Simon, U. John and M. M. Lerch (2005). "Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence." Digestion **71**(2): 97-105.

Voronina, S., R. Longbottom, R. Sutton, O. H. Petersen and A. Tepikin (2002). "Bile acids induce calcium signals in mouse pancreatic acinar cells: implications for bile-induced pancreatic pathology." J Physiol **540**(Pt 1): 49-55.

Voronina, S., T. Sukhomlin, P. R. Johnson, G. Erdemli, O. H. Petersen and A. Tepikin (2002). "Correlation of NADH and Ca2+ signals in mouse pancreatic acinar cells." J Physiol **539**(Pt 1): 41-52.

Voronina, S. G., S. L. Barrow, O. V. Gerasimenko, O. H. Petersen and A. V. Tepikin (2004). "Effects of secretagogues and bile acids on mitochondrial membrane potential of pancreatic acinar cells: comparison of different modes of evaluating DeltaPsim." J Biol Chem **279**(26): 27327-27338.

Voronina, S. G., S. L. Barrow, A. W. Simpson, O. V. Gerasimenko, G. da Silva Xavier, G. A. Rutter, O. H. Petersen and A. V. Tepikin (2010). "Dynamic changes in cytosolic and mitochondrial ATP levels in pancreatic acinar cells." Gastroenterology **138**(5): 1976-1987.

Voronina, S. G., O. V. Gryshchenko, O. V. Gerasimenko, A. K. Green, O. H. Petersen and A. V. Tepikin (2005). "Bile acids induce a cationic current, depolarizing pancreatic acinar cells and increasing the intracellular Na+ concentration." J Biol Chem **280**(3): 1764-1770.

Wagner, A. C., H. Weber, L. Jonas, H. Nizze, M. Strowski, F. Fiedler, H. Printz, H. Steffen and B. Goke (1996). "Hyperthermia induces heat shock

protein expression and protection against cerulein-induced pancreatitis in rats." Gastroenterology **111**(5): 1333-1342.

Walker, N. I., C. M. Winterford and J. F. Kerr (1992). "Ultrastructure of the rat pancreas after experimental duct ligation. II. Duct and stromal cell proliferation, differentiation, and deletion." Pancreas **7**(4): 420-434.

Walsh, C. T., L. D. Zydowsky and F. D. McKeon (1992). "Cyclosporin A, the cyclophilin class of peptidylprolyl isomerases, and blockade of T cell signal transduction." J Biol Chem **267**(19): 13115-13118.

Walter, P. and D. Ron (2011). "The unfolded protein response: from stress pathway to homeostatic regulation." Science **334**(6059): 1081-1086.

Wan, M. H., J. Li, H. L. Gong, P. Xue, L. Zhu, G. Y. Chen, Q. Xia and T. Wen-Fu (2011). "Clinical observation on the effect of dexamethasone and Chinese herbal decoction for purgation in severe acute pancreatitis patients." Chin J Integr Med **17**(2): 141-145.

Wang, G., J. Wen, R. R. Wilbur, P. Wen, S. F. Zhou and X. Xiao (2013). "The effect of somatostatin, ulinastatin and Salvia miltiorrhiza on severe acute pancreatitis treatment." Am J Med Sci **346**(5): 371-376.

Wang, J., G. Chen, H. Gong, W. Huang, D. Long and W. Tang (2012). "Amelioration of experimental acute pancreatitis with Dachengqi Decoction via regulation of necrosis-apoptosis switch in the pancreatic acinar cell." PLoS One **7**(7): e40160.

Wang, J., J. Su, Y. Lu, H. Zhou and B. Gong (2014). "A randomized control study to investigate the application of Ulinastatin-containing contrast medium to prevent post-ERCP pancreatitis." Hepatogastroenterology **61**(136): 2391-2394.

Wang, P. and J. Heitman (2005). "The cyclophilins." Genome Biol **6**(7): 226.

Wang, Q., F. Jiao, P. Zhang, J. Yan, Z. Zhang, F. He, Q. Zhang, Z. Lv, X. Peng, H. Cai and B. Tian (2017). "CDK5-Mediated Phosphorylation-Dependent Ubiquitination and Degradation of E3 Ubiquitin Ligases GP78 Accelerates Neuronal Death in Parkinson's Disease." Mol Neurobiol.

Wang, R., F. Yang, H. Wu, Y. Wang, Z. Huang, B. Hu, M. Zhang and C. Tang (2013). "High-dose versus low-dose octreotide in the treatment of acute pancreatitis: a randomized controlled trial." Peptides **40**: 57-64.

Wang, X., Y. Carlsson, E. Basso, C. Zhu, C. I. Rousset, A. Rasola, B. R. Johansson, K. Blomgren, C. Mallard, P. Bernardi, M. A. Forte and H. Hagberg (2009). "Developmental shift of cyclophilin D contribution to hypoxic-ischemic brain injury." J Neurosci **29**(8): 2588-2596.

Wang, X., W. Li, C. Niu, L. Pan, N. Li and J. Li (2011). "Thymosin alpha 1 is associated with improved cellular immunity and reduced infection rate in severe acute pancreatitis patients in a double-blind randomized control study." Inflammation **34**(3): 198-202.

Wang, X., X. Zeng, B. Yang, S. Zhao, W. Chen and X. Guo (2015). "Efficacy of thymosin alpha1 and interferon alpha for the treatment of severe acute pancreatitis in a rat model." Mol Med Rep **12**(5): 6775-6781.

Wang, Z., H. Jiang, S. Chen, F. Du and X. Wang (2012). "The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways." Cell **148**(1-2): 228-243.

Wang, Z. F., C. Liu, Y. Lu, R. Dong, J. Xu, L. Yu, Y. M. Yao, Q. G. Liu and C. E. Pan (2004). "Dexamethasone and dextran 40 treatment of 32 patients with severe acute pancreatitis." World J Gastroenterol **10**(9): 1333-1336.

Ward, J. B., R. Sutton, S. A. Jenkins and O. H. Petersen (1996). "Progressive disruption of acinar cell calcium signaling is an early feature of cerulein-induced pancreatitis in mice." Gastroenterology **111**(2): 481-491.

Warne, J., G. Pryce, J. M. Hill, X. Shi, F. Lenneras, F. Puentes, M. Kip, L. Hilditch, P. Walker, M. I. Simone, A. W. Chan, G. J. Towers, A. R. Coker, M. R. Duchen, G. Szabadkai, D. Baker and D. L. Selwood (2016). "Selective Inhibition of the Mitochondrial Permeability Transition Pore Protects against Neurodegeneration in Experimental Multiple Sclerosis." J Biol Chem **291**(9): 4356-4373.

Warzecha, Z., P. Ceranowicz, A. Dembinski, J. Cieszkowski, B. Kusnierz-Cabala, R. Tomaszewska, A. Kuwahara and I. Kato (2010). "Therapeutic effect of ghrelin in the course of cerulein-induced acute pancreatitis in rats." J Physiol Pharmacol **61**(4): 419-427.

Warzecha, Z., A. Dembinski, P. C. Konturek, P. Ceranowicz and S. J. Konturek (1999). "Epidermal growth factor protects against pancreatic damage in cerulein-induced pancreatitis." Digestion **60**(4): 314-323.

Weber, C. K., T. Gress, F. Muller-Pillasch, M. M. Lerch, H. Weidenbach and G. Adler (1995). "Supramaximal secretagogue stimulation enhances heat shock protein expression in the rat pancreas." Pancreas **10**(4): 360-367.

Weber, H., A. C. Wagner, L. Jonas, J. Merkord, T. Hofken, H. Nizze, P. Leitzmann, B. Goke and P. Schuff-Werner (2000). "Heat shock response is associated with protection against acute interstitial pancreatitis in rats." Dig Dis Sci **45**(11): 2252-2264.

Wen, L., S. Voronina, M. A. Javed, M. Awais, P. Szatmary, D. Latawiec, M. Chvanov, D. Collier, W. Huang, J. Barrett, M. Begg, K. Stauderman, J. Roos, S. Grigoryev, S. Ramos, E. Rogers, J. Whitten, G. Velicelebi, M. Dunn, A. V. Tepikin, D. N. Criddle and R. Sutton (2015).

"Inhibitors of ORAI1 Prevent Cytosolic Calcium-Associated Injury of Human Pancreatic Acinar Cells and Acute Pancreatitis in 3 Mouse Models." Gastroenterology **149**(2): 481-492 e487.

Wenhong, D., Y. Jia, W. Weixing, C. Xiaoyan, C. Chen, X. Sheng and J. Hao (2012). "Zerumbone attenuates the severity of acute necrotizing pancreatitis and pancreatitis-induced hepatic injury." Mediators Inflamm **2012**: 156507.

Werner, J., M. Laposata, C. Fernandez-del Castillo, M. Saghir, R. V. Iozzo, K. B. Lewandrowski and A. L. Warshaw (1997). "Pancreatic injury in rats induced by fatty acid ethyl ester, a nonoxidative metabolite of alcohol." Gastroenterology **113**(1): 286-294.

Werner, J., M. Saghir, A. L. Warshaw, K. B. Lewandrowski, M. Laposata, R. V. Iozzo, E. A. Carter, R. J. Schatz and C. Fernandez-Del Castillo (2002). "Alcoholic pancreatitis in rats: injury from nonoxidative metabolites of ethanol." Am J Physiol Gastrointest Liver Physiol **283**(1): G65-73.

Westermann, B. (2010). "Mitochondrial fusion and fission in cell life and death." Nat Rev Mol Cell Biol **11**(12): 872-884.

Whitcomb, D. C. (2013). "Genetic risk factors for pancreatic disorders." Gastroenterology **144**(6): 1292-1302.

Whitcomb, D. C., J. LaRusch, A. M. Krasinskas, L. Klei, J. P. Smith, R. E. Brand, J. P. Neoptolemos, M. M. Lerch, M. Tector, B. S. Sandhu, N. M. Guda, L. Orlichenko, C. Alzheimer's Disease Genetics, S. Alkaade, S. T. Amann, M. A. Anderson, J. Baillie, P. A. Banks, D. Conwell, G. A. Cote, P. B. Cotton, J. DiSario, L. A. Farrer, C. E. Forsmark, M. Johnstone, T. B. Gardner, A. Gelrud, W. Greenhalf, J. L. Haines, D. J. Hartman, R. A. Hawes, C. Lawrence, M. Lewis, J. Mayerle, R. Mayeux, N. M. Melhem, M. E. Money, T. Muniraj, G. I. Papachristou, M. A. Pericak-Vance, J. Romagnuolo, G. D. Schellenberg, S. Sherman, P. Simon, V. P. Singh, A. Slivka, D. Stolz, R.

Sutton, F. U. Weiss, C. M. Wilcox, N. O. Zarnescu, S. R. Wisniewski, M. R. O'Connell, M. L. Kienholz, K. Roeder, M. M. Barmada, D. Yadav and B. Devlin (2012). "Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis." Nat Genet **44**(12): 1349-1354.

Wiederrecht, G., S. Hung, H. K. Chan, A. Marcy, M. Martin, J. Calaycay, D. Boulton, N. Sigal, R. L. Kincaid and J. J. Siekierka (1992). "Characterization of high molecular weight FK-506 binding activities reveals a novel FK-506-binding protein as well as a protein complex." J Biol Chem **267**(30): 21753-21760.

Windsor, J. A., K. C. Fearon, J. A. Ross, G. R. Barclay, E. Smyth, I. Poxton, O. J. Garden and D. C. Carter (1993). "Role of serum endotoxin and antiendotoxin core antibody levels in predicting the development of multiple organ failure in acute pancreatitis." Br J Surg **80**(8): 1042-1046.

Wittel, U. A., T. Wiech, S. Chakraborty, B. Boss, R. Lauch, S. K. Batra and U. T. Hopt (2008). "Taurocholate-induced pancreatitis: a model of severe necrotizing pancreatitis in mice." Pancreas **36**(2): e9-21.

Working Group, I. A. P. A. P. A. A. P. G. (2013). "IAP/APA evidence-based guidelines for the management of acute pancreatitis." Pancreatology **13**(4 Suppl 2): e1-15.

Wu, B. U., R. S. Johannes, S. Kurtz and P. A. Banks (2008). "The impact of hospital-acquired infection on outcome in acute pancreatitis." Gastroenterology **135**(3): 816-820.

Wu, J. S., W. M. Li, Y. N. Chen, Q. Zhao and Q. F. Chen (2016). "Endoplasmic reticulum stress is activated in acute pancreatitis." J Dig Dis **17**(5): 295-303.

Wu, Q. F., C. Qian, N. Zhao, Q. Dong, J. Li, B. B. Wang, L. Chen, L. Yu, B. Han, Y. M. Du and Y. H. Liao (2017). "Activation of transient receptor potential vanilloid 4 involves in hypoxia/reoxygenation injury in cardiomyocytes." Cell Death Dis **8**(5): e2828.

Yadav, D. and A. B. Lowenfels (2013). "The epidemiology of pancreatitis and pancreatic cancer." Gastroenterology **144**(6): 1252-1261.

Yamaguchi, R., A. Andreyev, A. N. Murphy, G. A. Perkins, M. H. Ellisman and D. D. Newmeyer (2007). "Mitochondria frozen with trehalose retain a number of biological functions and preserve outer membrane integrity." Cell Death Differ **14**(3): 616-624.

Yamanel, L., M. R. Mas, B. Comert, A. T. Isik, S. Aydin, N. Mas, S. Deveci, M. Ozyurt, I. Tasci and T. Unal (2005). "The effect of activated protein C on experimental acute necrotizing pancreatitis." Crit Care **9**(3): R184-190.

Yang, C., F. Guanghua, Z. Wei, J. Zhong, J. Penghui, F. Xin and Z. Xiping (2010). "Combination of hemofiltration and peritoneal dialysis in the treatment of severe acute pancreatitis." Pancreas **39**(1): 16-19.

Yang, F., H. Wu, Y. Li, Z. Li, C. Wang, J. Yang, B. Hu, Z. Huang, R. Ji, X. Zhan, H. Xie, L. Wang, M. Zhang and C. Tang (2012). "Prevention of severe acute pancreatitis with octreotide in obese patients: a prospective multi-center randomized controlled trial." Pancreas **41**(8): 1206-1212.

Yasar, M., B. Uysal, U. Kaldirim, Y. Oztas, S. Sadir, M. Ozler, T. Topal, O. Coskun, A. Kilic, T. Cayci, Y. Poyrazoglu, S. Oter, A. Korkmaz and A. Guven (2010). "Poly(ADP-ribose) polymerase inhibition modulates experimental acute necrotizing pancreatitis-induced oxidative stress, bacterial translocation and neopterin concentrations in rats." Exp Biol Med (Maywood) **235**(9): 1126-1133.

Ye, R., O. A. Mareninova, E. Barron, M. Wang, D. R. Hinton, S. J. Pandol and A. S. Lee (2010). "Grp78 heterozygosity regulates chaperone balance in exocrine pancreas with differential response to cerulein-induced acute pancreatitis." Am J Pathol **177**(6): 2827-2836.

Yoo, J. W., J. K. Ryu, S. H. Lee, S. M. Woo, J. K. Park, W. J. Yoon, J. K. Lee, K. H. Lee, J. H. Hwang, Y. T. Kim and Y. B. Yoon (2008). "Preventive effects of ulinastatin on post-endoscopic retrograde cholangiopancreatography pancreatitis in high-risk patients: a prospective, randomized, placebo-controlled trial." Pancreas **37**(4): 366-370.

Yoo, K. S., K. R. Huh, Y. J. Kim, K. O. Kim, C. H. Park, T. Hahn, S. H. Park, J. H. Kim, C. K. Park, Y. J. Kwon and G. A. Lehman (2011). "Nafamostat mesilate for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis: a prospective, randomized, double-blind, controlled trial." Pancreas **40**(2): 181-186.

You, N., K. Tao, W. Zhao, P. Bao, R. Li, H. You, M. Zhang, Z. Gao, Z. Gao and K. Dou (2010). "Diphenhydramine modulates cytokines and induces apoptosis in experimental acute pancreatitis." Methods Find Exp Clin Pharmacol **32**(5): 311-318.

Youle, R. J. and D. P. Narendra (2011). "Mechanisms of mitophagy." Nat Rev Mol Cell Biol **12**(1): 9-14.

Yu, G., R. Wan, Y. Hu, J. Ni, G. Yin, M. Xing, J. Shen, M. Tang, C. Chen, Y. Fan, W. Xiao, Y. Zhao, X. Wang and G. Hu (2014). "Pancreatic acinar cells-derived cyclophilin A promotes pancreatic damage by activating NF-kappaB pathway in experimental pancreatitis." Biochem Biophys Res Commun **444**(1): 75-80.

Yuasa, C., K. Irimura and T. Oka (1998). "Ethanol administration delays recovery from acute pancreatitis induced by exocrine hyperstimulation." J Pharmacol Toxicol Methods **39**(4): 221-228.

Zaninovic, V., A. S. Gukovskaya, I. Gukovsky, M. Mouria and S. J. Pandol (2000). "Cerulein upregulates ICAM-1 in pancreatic acinar cells, which mediates neutrophil adhesion to these cells." Am J Physiol Gastrointest Liver Physiol **279**(4): G666-676.

Zhang, M. J., G. L. Zhang, W. B. Yuan, J. Ni and L. F. Huang (2008). "Treatment of abdominal compartment syndrome in severe acute pancreatitis patients with traditional Chinese medicine." World J Gastroenterol **14**(22): 3574-3578.

Zhu, Y., X. Pan, H. Zeng, W. He, L. Xia, P. Liu, Y. Zhu, Y. Chen and N. Lv (2017). "A Study on the Etiology, Severity, and Mortality of 3260 Patients With Acute Pancreatitis According to the Revised Atlanta Classification in Jiangxi, China Over an 8-Year Period." Pancreas **46**(4): 504-509.