FACTORS INFLUENCING THE SEVERITY OF ACUTE PANCREATITIS: EXPERIMENTAL AND CLINICAL STUDIES

Ph.D. Thesis

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I. PUBLICATIONS

I.1. Publications related to the subject of the thesis

Fűr G, Bálint ER, Orján EM, Balla Z, Kormányos ES, Czira B, Szűcs A, Kovács DP, Pallagi P, Maléth J, Venglovecz V, Hegyi P, Kiss L, Rakonczay Z Jr. Mislocalization of CFTR expression in acute pancreatitis and the beneficial effects of VX-661/VX-770 treatment on disease severity. J Physiol. 599, 4955-4971. doi: 10.1113/JP281765. IF2020: 5.182, Q1, D1

Bálint ER, **Fűr G**, Kui B, Balla Z, Kormányos ES, Orján EM, Tóth B, Horváth G, Szűcs E, Benyhe S, Ducza E, Pallagi P, Maléth J, Venglovecz V, Hegyi P, Kiss L, Rakonczay Z Jr. Fentanyl but not morphine or buprenorphine improves the severity of necrotizing acute pancreatitis in rats. Int J Mol Sci. 23, 1192. doi: 10.3390/ijms23031192. IF2020-2021: 5.924, Q1, D1

Kiss L*, **Fűr G***, Mátrai P, Hegyi P, Iványi E, Cazacu IM, Szabó I, Habon T, Alizadeh H, Gyöngyi Z, Vigh É, Erőss B, Erős A, Ottoffy M, Czakó L, Rakonczay Z Jr. The effect of serum triglyceride concentration on the outcome of acute pancreatitis: systematic review and meta-analysis. Sci Rep. 8, 14096. doi: 10.1038/s41598-018-32337-x. IF2018: 4.011, Q1, D1
*Authors share a co-first authorship

I.2. Publications not related to the subject of the thesis

Balla Z, Kormányos ES, Kui B, Bálint ER, **Fűr G**, Orján EM, Iványi B, Vécsei L, Fülöp F, Varga G, Harazin A, Tubak V, Deli MA, Papp C, Gácser A, Madácsy T, Venglovecz V, Maléth J, Hegyi P, Kiss L, Rakonczay Z Jr. Kynurenic acid and its analogue SZR-72 ameliorate the severity of experimental acute necrotizing pancreatitis. Front Immunol. 12, 702764. doi: 10.3389/fimmu.2021.702764. IF2020: 7.561, Q1

Gróf I, Bocsik A, Harazin A, Santa-Maria AR, Vizsnyiczai G, Barna L, Kiss L, **Fűr G**, Rakonczay Z Jr, Ambrus R, Szabó-Révész P, Gosselet F, Jaikumpun P, Szabó H, Zsembery Á, Deli MA. The effect of sodium bicarbonate, a beneficial adjuvant molecule in cystic fibrosis, on bronchial epithelial cells expressing a wild-type or mutant CFTR channel. Int J Mol Sci. 21, 4024. doi: 10.3390/ijms21114024. IF2020: 5.923, Q1, D1

Bálint ER, **Fűr G**, Kiss L, Németh DI, Soós A, Hegyi P, Szakács Z, Tinusz B, Varjú P, Vincze Á, Erőss B, Czimmer J, Szepes Z, Varga G, Rakonczay Z Jr. Assessment of the course of acute pancreatitis in the light of aetiology: a systematic review and meta-analysis. Sci Rep. 10, 17936. doi: 10.1038/s41598-020-74943-8. IF2020: 4.379, Q1, D1

Szakács Z, Hegyi PJ, Farkas N, Hegyi P, Balaskó M, Erős A, Szujó S, Pammer J, Mosdósi B, Simon M, Nagy A, **Fűr G**, Hussain A. Pregnancy outcomes of women whom spouse fathered children after tyrosine kinase inhibitor therapy for chronic myeloid leukemia: A systematic review. PLoS One. 15, e0243045. doi: 10.1371/journal.pone.0243045. IF2020: 2.740, Q1, D1

I.3. Scientometrics

Number of publications:	7
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II. LIST OF ABBREVATIONS

AP- acute pancreatitis

BCECF-AM- 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester

CA- carbonic anhydrase

cAMP- cyclic adenosine monophosphate

Cer- cerulein

Cer-AP- Cer-induced AP

CFTR- cystic fibrosis transmembrane conductance regulator

CK19- cytokeratin-19

DMSO- dimethyl sulfoxide

ERCP- endoscopic retrograde cholangiopancreatography

FE- fentanyl

FFA- free fatty acid

H&E- hematoxylin and eosin

HTG- hypertriglyceridemia

HTG-AP- hypertriglyceridemia-induced acute pancreatitis

ICU- intensive care unit

i.d.- intraductal

i.p.- intraperitoneal

LO- L-ornithine

MOF- multi-organ failure

MPO- myeloperoxidase

NaTc- sodium-taurocholate

NaTc-AP- NaTc-induced AP

NBC- Na^+/ HCO_3^- co-transporter

NEFA- non-esterified fatty acids

NHE- Na^+/H^+ exchanger

NOS- Newcastle–Ottawa Scale

NTGAP- non-TG-related AP

pH_i- intracellular pH

PICO- Problem, Intervention, Comparison intervention, and Outcome

POF- persistent organ failure

seTG- serum triglyceride

SLC26- solute carrier family 26

TBS- tris-buffered solution

TGAP- TG-related AP

TOF- transient organ failure

VX-661- tezacaftor

VX-770- ivacaftor

III. INTRODUCTION

III.1. The physiological functions of the pancreas

The pancreas consists of endocrine and exocrine parts. The endocrine cells are responsible for the production of various hormones such as insulin and glucagon. The exocrine pancreas mainly consists of acinar and ductal cells (Czakó et al., 2009). The acinar cells secrete inactive digestive enzymes in a NaCl-rich isotonic fluid (Figure 1.). The pancreatic ductal epithelium secretes 2.5 litres of alkaline fluid daily that may contain up to 140 mM HCO_3^- (Hegyi & Rakonczay, 2015). Furthermore, the ductal cells provide structural framework for pancreas, convey digestive proenzymes secreted by acini, neutralize the acidic fluid of the acini as well as the gastric juice entering the duodenum (Pallagi et al., 2015; Zeng M et al., 2017).

The ductal cells are responsible for secretion of fluid and HCO_3^- in two steps (Lee et al., 2012) (Figure 1.). Secretion is mainly regulated by the gastrointestinal hormone secretin (Pallagi et al., 2014). The rate of ductal fluid production is closely correlated with the amount secreted HCO_3^-. The intracellular accumulation and extracellular secretion of HCO_3^- can occur by both passive and active pathways. The passive step is diffusion of CO_2, while the active mechanism is via ion channels and transporters. CO_2 diffuses into the cells from the blood and carbonic anhydrase (CA) converts it to HCO_3^-. Basolateral transporters like Na^+/HCO_3^- co-transporter (NBC), Na^+/H^+ exchanger (NHE) or H^+-ATPase contribute to HCO_3^- uptake into ductal cells (Ishiguro et al., 2012). The secretion of HCO_3^- across the apical membrane into the lumen of the ducts occurs mainly via four anion channels and transporters: cystic fibrosis transmembrane conductance regulator (CFTR), solute carrier family 26 (SLC26) anion exchangers (DRA and SLC26A6) and Anoctamin-1 (Ishiguro et al., 2007 and 2012; Saint-Criq & Gray, 2017). Among these ion transporters, CFTR is the most prominent due to its numerous roles in secretion (fluid, Cl^- and HCO_3^-) and regulation of other ion channels, like SLC26A6 (Hegyi et al., 2016; Kim et al., 2020; Rakonczay et al., 2008). Therefore, CFTR functions as a signalling hub.

The Cl^- conductance of CFTR in the apical membrane is largely determined by the activity of the channel, i.e., its ability to open, which is regulated by the phosphorylation of cAMP/protein kinase A, the amount and conductance of CFTR channels, the latter of which is regulated by the electrochemical gradient across the apical membrane (Saint-Criq & Gray, 2017). CFTR alters Ca^{2+} signalling through direct connection with sarco-/endoplasmic reticulum Ca^{2+}-ATPase and plasma membrane Ca^{2+}-ATPase channels (Philippe et al., 2015) or by functional coupling with transient receptor potential canonical 6 (Antigny et al., 2008).

Moreover, the presence of CFTR is necessary for proper function of mitochondria (Madácsy et al., 2018).

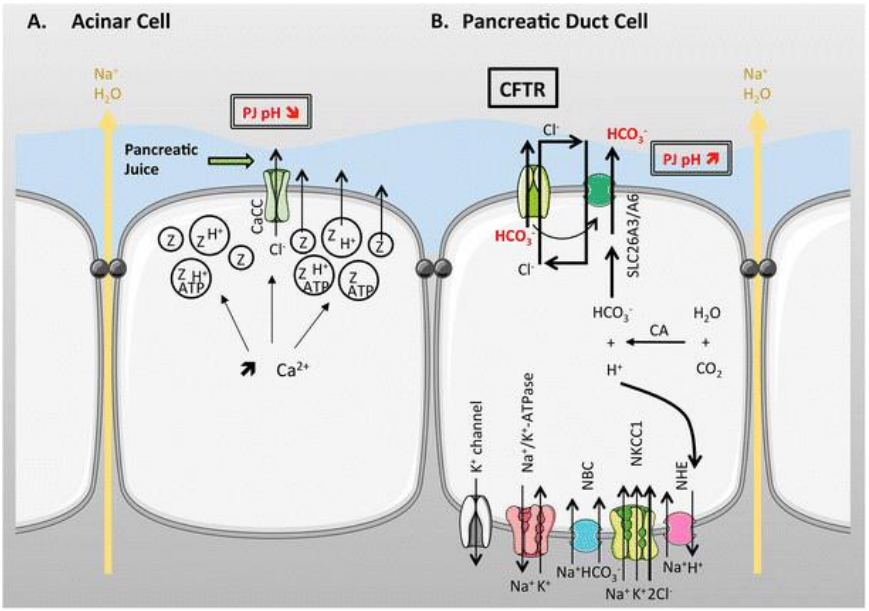

Figure 1. Ion transport in acinar and pancreatic duct cells. PJ, pancreatic juice; CaCC, calcium-activated Cl^- channel; Z, zymogens; ATP, adenosine triphosphate; CFTR, cystic fibrosis transmembrane conductance regulator; SLC26A3, solute carrier family 26A3; SLC26A6, solute carrier family 26A6; CA, carbonic anhydrase; NBC, Na^+ /HCO_3^- cotransporter; NKCC1, $Na^+/K^+/2Cl^-$ co-transporter; NHE, Na^+/H^+ exchanger. Source: Saint-Criq & Gray, 2017.

III.2. Acute pancreatitis

III.2.1. Epidemiology and diagnostic criteria

Acute pancreatitis (AP) is the sudden inflammation of the pancreas and one of the most common gastrointestinal diseases requiring hospitalisation. (Peery et al., 2015). Its incidence shows increasing tendency, and it is more than 30 per 100 000 population in Europe (Roberts et al., 2013 and 2017). The diagnosis of AP is a combination of both subjective and objective findings (Olson et al., 2019). This includes epigastric upper abdominal pain, more than 3 times elevated serum or urinary amylase/lipase activity, and imaging consistent with the diagnosis (García-Rayado et al., 2020; Olson et al., 2019). Meeting two of these three criteria helps ensure appropriate diagnosis (Banks et al., 2013; Hritz et al., 2015; Olson et al., 2019). Notably, pain is present in 90-95% of AP patients (Olson et al., 2019; Párniczky et al., 2016). The severity of the disease can be classified into three groups based on the modified Atlanta criteria: mild, moderately severe and severe (Banks et al., 2013). The appearance of organ failure determines the severity of the disease (Banks et al., 2013). Patients in the mild group have no organ disfunction, whereas in the moderate and severe groups there is transient organ failure (TOF, < 48 h) or persistent organ failure (POF, > 48 h), respectively (Schepers et al., 2019).

III.2.2. Etiological factors

Massive alcohol consumption and gallstone disease are responsible for about 60–80% of AP cases, whereas 1–9% of the cases are hypertriglyceridemia (HTG)-induced (Carr et al., 2016; Forsmark et al., 2016; Yadav & Lowenfels, 2013). Biliary pancreatitis can develop from a gallstone caused blockage of either the bile duct or the pancreatic duct (Wang GJ et al., 2009). Patients with alcohol-induced AP are characterized by regular and high volume of alcohol consumption (50-100 g/day), with clinical follow-up over 5 years (Yadav & Whitcomb, 2010). The aetiology of HTG can be primary (caused by genetic mutations), but it is most commonly secondary (Tsuang et al., 2009). The causes of secondary HTG include obesity, unhealthy diet or lifestyle, pregnancy, hypothyroidism, hepatic steatosis, nephrotic syndrome, type-2 diabetes mellitus, and intake of certain drugs (e.g. glucocorticoids, oestrogen or tamoxifen) (Reiner, 2017; Shah and Wilson, 2015; Zhang et al., 2019). HTG-induced AP (HTG-AP) is generally related to recurrent attacks of AP (Guo et al., 2019; Kilinc et al., 2018). Patients with HTG-AP are characterized by younger age and predominantly male gender, but higher fat intake or higher BMI may also contribute to this aetiology (Adiamah et al., 2017; Wang L et al., 2021). Furthermore, complications or the need for hospitalisation seem to be more common in HTG-AP than in other aetiologies (Wang L et al., 2021). Moreover, morbidity (such as renal failure, shock, and infections) and mortality are reported to be significantly higher in patients with HTG-AP than in AP patients of other pathologies (Rawla et al., 2018). Interestingly, in pregnancy, up to 56% of AP cases are HTG-related (Tsuang et al., 2009).

III.2.2.1. Effect of serum triglyceride levels on disease severity

The reference value of triglyceride (TG) in the blood serum is below 1.7 mM (Santos-Baez and Ginsberg, 2020). The extent of HTG has been classified by the Endocrine Society into the following groups based on fasting serum TG (seTG): mild (1.7 to 2.3 mM), moderate (2.3 to 11.2 mM), severe (11.3 to 22.4 mM) and very severe HTG (>22.4 mM) (Berglund et al., 2012). It is widely accepted that severe or very severe HTG (>11.3mM) markedly increases the risk for AP (Fortson et al., 1995; Tsuang et al., 2009). However, some authors define HTG-AP when seTG is >5.6 mM (Carr et al., 2016). There is no significant evidence for HTG-AP at <5.6 mM seTG (Gelrud & Whitcomb, 2016). Beyond the increased risk for AP in severe HTG, previous publications have indicated that there is a relationship between seTG and the severity of AP (Carr et al., 2016; Murad et al., 2012; Wang Q et al., 2017), even in the case of mild or moderate HTG (Nawaz et al., 2015; Sue et al., 2017; Wan et al., 2017; Zeng Y et al., 2014). Nawaz et al. (2015) reported that HTG independently and proportionally correlates with persistent organ failure regardless of AP aetiology. Zeng Y et al. (2014) showed that seTG

>2.26 mM increases the risk for systemic and local complications in acute biliary pancreatitis. However, some studies have shown no relationship between seTG and the severity of AP (Balachandra et al., 2006; Gubensek et al., 2014). These discrepancies urge further analyses of the effect of seTG on the severity of AP.

III.2.3. Pathomechanism

The mechanisms underlying the development of AP are complex and not fully understood. AP pathogenesis includes toxic intracellular Ca^{2+} overload which induces premature activation of digestive enzymes, activation of the nuclear factor kappa B, impairment of autophagy, mitochondrial dysfunction, as well as impairment of ductal function (Barreto et al., 2021). The aetiology of the disease can determine the pathogenesis of AP.

III.2.3.1 Impact of aetiological factors

In biliary-AP, gallstones can increase pressure in the ductal lumen, prevent proper discharge of digestive enzymes and subsequently trigger their uncontrolled and premature activation, promoting the development of inflammation (Diehl et al., 1997). In alcohol-induced AP, alcohol triggers several biochemical changes in pancreatic cells (Clemens et al., 2016). These include the existence of persistent intracellular calcium levels, activation of the mitochondrial permeability transition pore, endoplasmic reticulum stress, disruption of autophagy, alteration in activity of transcriptional activators, and colocalization of lysosomal and pancreatic digestive enzymes.

The underlying mechanism by which HTG exacerbates the severity of AP is unknown. One of the possible processes is that pancreatic lipases metabolize seTG to non-esterified fatty acids (NEFA) (Pedersen et al., 2016; Valdivielso et al., 2014). These NEFA are toxic and cause MOF to worsen the outcome of AP (Durgampudi et al., 2014; Navina et al., 2011; Petersen et al., 2009; Sztefko & Panek, 2001). The administration of NEFA induced sustained elevation of Ca^{2+} concentration in pancreatic acinar cells and inhibited mitochondrial function and ATP production (Criddle et al., 2006; Maléth et al., 2013). Consequently, NEFAs cause damage to acinar and vascular endothelial cells, thus leading to inflammation (Patel et al., 2016). Our earlier studies have also indicated that fatty acids inhibit CFTR activity and decrease the HCO_3^- and fluid secretion of pancreatic ductal cells (Judák et al., 2014; Maléth et al., 2015). In addition, in the case of HTG, the concentration of chylomicrons is elevated. This increases blood viscosity, which impairs blood flow and results in pancreatic ischemia and acidosis (Pedersen et al., 2016; Valdivielso et al., 2014) (Figure 2.).

III.2.3.2. The role of ducts and CFTR

Pancreatic ductal cell damage can occur during AP which is associated with impaired ductal secretion. If ducts do not function, pancreatic acinar cell protection is reduced and activated enzymes cannot be appropriately eliminated from the pancreas. There are different factors which affect ductal activity. Activated trypsin in ductal lumen inhibits CFTR via proteinase-activated receptor 2 (Pallagi et al., 2011). Moreover, the impaired ductal function due to CFTR mutations in cystic fibrosis (Judák et al., 2014; Maléth et al., 2011 and 2015; Venglovecz et al., 2008) or CFTR inhibition in AP decreases bicarbonate and fluid secretion (Hegyi et al., 2011; Sahin-Tóth & Hegyi, 2017; Tóth et al., 2019) and lead to aggravated AP.

CFTR activity contributes significantly to proper channel function. Disturbance or loss of ductal function is a key factor in the development of AP. Several drugs have recently been clinically approved to improve CFTR expression, localisation and function by correcting the folding of the protein or potentiating its activity in cystic fibrosis (Amaral, 2021). Of these, the CFTR corrector lumacaftor (VX-809), tezacaftor (VX-661), elexacaftor (VX-445) and the CFTR potentiator ivacaftor (VX-770) have been shown to be the most effective.

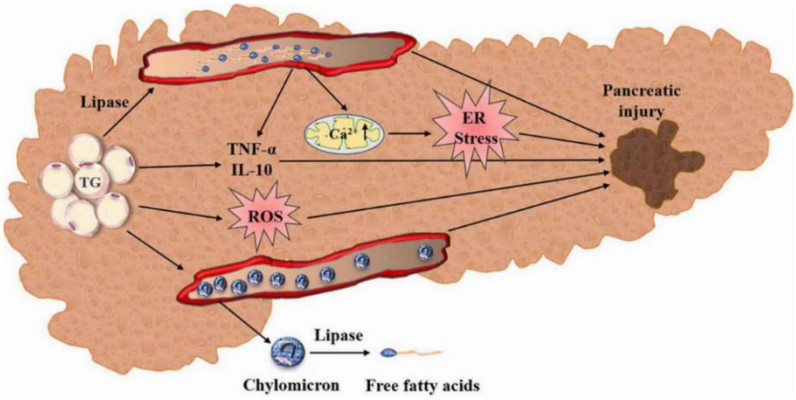

Figure 2. Schematic diagram showing our current understanding of the pathogenesis of hypertriglyceridemia (HTG)-induced acute pancreatitis (AP) (HTG-AP). TG, triglyceride; TNF-α, tumour necrosis factor alpha; IL-10, interleukin 10; ROS, reactive oxygen species; ER, endoplasmic reticulum. Source: Guo et al., 2019.

III.2.4. Treatment

The therapy of AP is only supportive and there is no specific drug against it (Crockett et al., 2018). Nowadays, the opportunities for early management of AP are analgesia, Ringer's lactate solution-based fluid resuscitation, and early oral refeeding, or if it is not tolerated enteral nutrition should be applied (Crockett et al., 2018; García-Rayado et al., 2020) (Figure 3.). Pain is typically managed with parenteral analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, and opioids (Machicado & Papachristou, 2019). Unfortunately, recent guidelines for AP treatment do not have clear recommendations for the types of analgesics to be used (Crockett et al., 2018; Working Group IAP/APA Acute Pancreatitis

Guidelines, 2013) and for the preferable administration route of pain relievers (García-Rayado et al., 2020). In an Italian study, 55% of patients were treated with NSAIDs and only 4% were given opioids, whereas in the United States 93% of patients were treated with opioids for AP-induced pain (Machicado & Papachristou, 2019). Although some randomized controlled trials have compared different analgesics in AP, most of them only included a few patients and had low methodological quality (García-Rayado et al., 2020; Machicado & Papachristou, 2019; Meng W et al., 2013). Classic NSAIDs and metamizole can be used to treat AP pain, although their adverse effects (gastrointestinal damage and renal impairment with NSAIDs and neutropenia with metamizole) must be considered (García-Rayado et al., 2020). Opioids, like fentanyl (FE), buprenorphine (BQ), pethidine, pentazocine, morphine (MO) are the most effective pain killers. There is a scientific debate on the use of opioids due to their side effects such as constipation or immunosuppression (Franchi et al., 2019). FE and MO administration is less preferred in humans due to the spasm of sphincter of Oddi which might worsen the outcome of AP (Afghani et al., 2017; Thompson, 2001). Interestingly, BQ has no morphine-like effect on the sphincter of Oddi (Cuer et al., 1989). Barlass and his colleagues have shown in wild-type BL/6 mice that MO exacerbates AP and impairs tissue regeneration (Barlass et al., 2018). However, a systematic review concluded that opioids might reduce the need for supplementary analgesics without increasing adverse effects (Basurto et al., 2013; García-Rayado et al., 2020). Different proposals for the remaining uncertainties can be found in the literature: epidural analgesia may be an alternative in AP patients with intense pain (García-Rayado et al., 2020), or non-opioid analgesics can be considered as the first line of analgesia in patients with AP (Machicado & Papachristou, 2019).

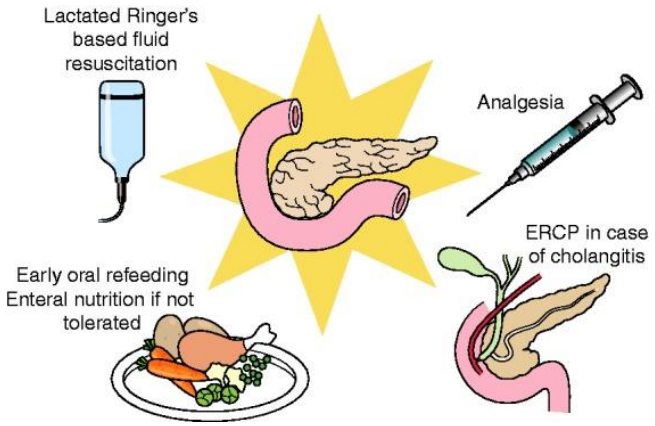

Figure 3. Early management of AP. ERCP, endoscopic retrograde cholangiopancreatography. Source: García-Rayado et al., 2020.

IV. AIMS

Our overall goal was to investigate the effects of different factors on AP by using experimental animal models or processing clinical data. Based on these, our detailed aims were the following:

a. to reveal how the disease course affects pancreatic ductal functions and the expression of proteins involved in HCO_3^- secretion
b. to investigate how correction and stimulation of CFTR affect the disease severity
c. to study the effect of the pain reliever fentanyl, on the severity of experimental biliary AP
d. to evaluate and compare the effects of normal or elevated seTG on the severity, mortality, and other complications of AP in humans

V. MATERIALS AND METHODS

V.1. Animal experiments

V.1.1. Ethics

Animal experiments were implemented in compliance with the European Union Directive 2010/63/EU and the Hungarian Government Decree 40/2013 (II.14.). Experiments were authorized by both local (University of Szeged) and national ethics committees (X/3355/2017 and X/3354/2017) for investigations involving animals. Mice were sacrificed via intraperitoneal (i.p.) injection of 200 mg/kg pentobarbital, whereas in case of rats the dose was 85 mg/kg (Bimeda MTC, Cambridge, Canada).

V.1.2. Solution and chemicals

All chemicals were obtained from Merck Life Science Kft. (Budapest, Hungary), unless otherwise stated. Cerulein (Cer) was acquired from Glentham Life Sciences (Corsham, United Kingdom); VX-661 (tezacaftor) and VX-770 (ivacaftor) were obtained from Cayman Chemical (Ann Arbor, MI, USA); amylase activity kit was procured from Diagnosticum (Budapest, Hungary); anti-CFTR antibody was purchased from Alomone Labs (Jerusalem, Israel); AlexaFluor488 goat anti-rabbit secondary antibody and Hoechst 33342 were from ThermoFisher Scientific (Waltham, MA, USA); anti-cytokeratin-19 antibody was obtained from Abcam (Cambridge; United Kingdom); RNA/cDNA kits: DreamTaq DNA Polymerase, DreamTaq™ Green Buffer, dNTP mix 25 mM, GeneRuler 100 bp DNA Ladder, TRIzol™ Plus RNA Purification Kit, UltraPure™ Ethidium Bromide, GeneRuler™ 1 kb Plus DNA Ladder, High-Capacity cDNA Reverse Transcription Kit and Luminaris Color HiGreen qPCR Master

Mix were obtained from ThermoFisher Scientific; 2.7-bis-(2-carboxyethyl)-5-(and-6-) carboxyfluorescein-acetoxymethylester (BCECF-AM) was purchased from Biotium (Fremont, CA, USA). Cer, VX-661 and VX-770 were dissolved in dimethyl sulfoxide (DMSO) and Cer was further diluted in physiological saline (PS) before injection. Ketamine and xylazine were obtained from CP-Pharma-Handelsgesellschaft MBH (Burgdorf, Germany). The solutions used for ductal (intracellular pH, pH_i, and fluid secretion) measurements and immunohistochemistry are presented in Table 1.

V.1.3. Animals

8-10 week-old male FVB/n mice from Charles River Laboratories Inc. (Wilmington, MA, USA) or female Wistar rats weighing 200-250 g were used for experiments. The animals were housed in the departmental animal facility at a constant room temperature of 24 °C with a 12 h light-dark cycle and were allowed *ad libitum* to water and standard laboratory chow (Biofarm, Zagyvaszántó, Hungary).

	Standard HEPES (mM)	Standard HCO_3^-/CO_2 (mM)	NH_4^+ in HCO_3^-/CO_2 (mM)	Tris-buffered saline (mM)
NaCl	140	115	95	150
KCl	5	5	5	-
$MgCl_2$	1	1	1	-
$CaCl_2$	1	1	1	-
HEPES	10	-	-	-
Glucose	10	10	10	-
$NaHCO_3$		25	25	-
NH_4Cl			20	-
Trizma base				50
pH	7.4	set by 5 % CO_2 and 95 % O_2 bubbling	set by 5 % CO_2 and 95 % O_2 bubbling	7.4

Table 1. The solutions used for ductal measurements and immunohistochemistry.

V.1.4. *In vivo* experiments: acute pancreatitis induction and treatments

Two different experimental setups were utilized in the Cer-induced mouse model (Figure 4). In the first part of the study, AP was evoked to reveal its effect on CFTR expression and ductal function (Figure 4A). In the second part of our work, CFTR corrector and potentiator

(VX-661 and VX-770, respectively) administration was combined with AP induction (Figure 4B).

Necrotizing AP was induced by hourly i.p. injection of 6 or 10 × 50 µg/kg Cer (5 µg/ml) in FVB/n mice as described previously (Pallagi et al., 2014). VX-661 and VX-770 were administered i.p. at 2 mg/kg once a day before and during AP. The doses of VX-661 and VX-770 were chosen based on the study by Zeng M et al. (2017). Control groups were given PS instead of Cer, and DMSO instead of VX compounds. Animals were sacrificed at 0, 6, 12, 24, 48, and 72 h when the effect of AP on ducts and CFTR were investigated. In case of VX-661+VX-770 combination, the first termination time was at 24 h and the second at 48 h.

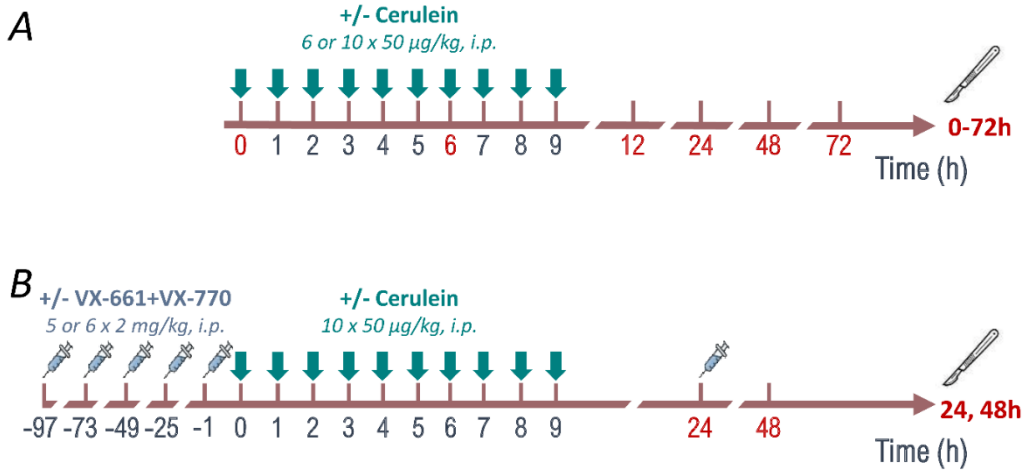

Figure 4. Schematic representation of experimental protocols. Experimental protocol for investigating A, the time course of cerulein (Cer)-induced AP (Cer-AP) severity in FVB/n mice; B, the effect of CFTR corrector (VX-661) and potentiator (VX-770) on AP. Arrows and syringe pictograms above the timeline indicate Cer and VX-661/VX-770 treatments, respectively. Control animals were injected with physiological saline (PS) instead of Cer and dimethyl sulfoxide (DMSO) instead of VX compounds. The scalper and red numbers indicate the times of sacrifice in h.

Necrotizing AP was induced by intraductal (i.d.) administration of 1 ml/kg Na-taurocholate solution (NaTc; 40 mg/ml) in rats as described previously (Pallagi et al., 2014). In case of NaTc -induced AP (NaTc-AP), abdominal surgery was performed on anaesthetized rats (with 70 mg/kg ketamine and 14 mg/kg xylazine i.p.). Then a cannula was placed into the pancreatic duct and the common bile duct was transiently occluded via a micro vessel clip. The NaTc solution was injected at a speed of 50 µL/min. At the end of the procedure, rats were placed on a heating pad for 40 mins or until they woke up. Thereafter, rats were placed back into their cages for 16-24 h. Control groups were given PS solution instead of NaTc, respectively. In case of NaTc-AP, rats were extensively monitored, and when body temperature decreased below 30 °C they were humanely sacrificed by deep anaesthesia induced by 85 mg/kg i.p. pentobarbital injection. Animals were sacrificed between 16-24 h.

FE was administered i.p. at doses of 0.1 and 0.2 mg/kg based on the literature data

(Almousa et al., 2011). FE was used as pre- or post-treatment in NaTc model. In the pre-treatment groups, the first FE injection was given 1 h prior to the induction of AP and it was repeated every 10 h in NaTc-AP (Figure 5). In preliminary experiments, FE pre-treatment was also tested in NaTc-AP, but the condition of animals was critical, therefore humane termination was performed and these investigations were stopped. In the post-treatment setup, since FE depresses respiration (Hill et al., 2020), it could not be administered within 3 h after surgery. Therefore, FE was injected 4 h after the NaTc model induction (Figure 5).

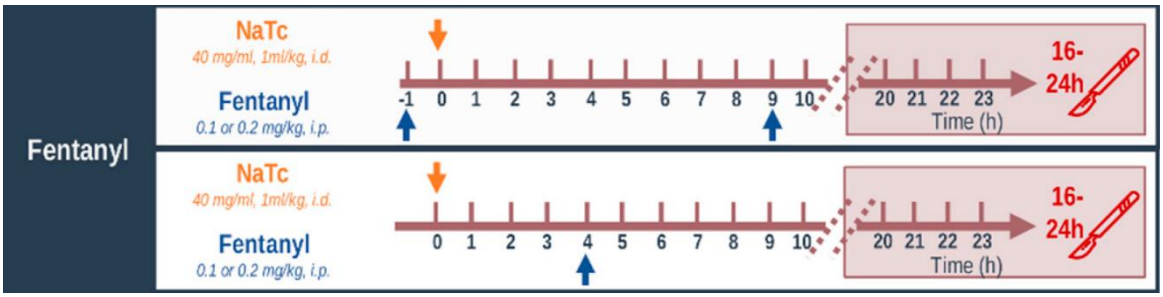

Figure 5. Schematic view of fentanyl (FE) treatment in Na-taurocholate (NaTc)-induced AP (NaTc-AP). Arrows above or below the timeline show the injections. Control animals were injected with PS. i.p., intraperitoneal; i.d., intraductal.

At the end of experiments, animals were sacrificed by pentobarbital injection. Immediately after opening the abdomen, a small piece of pancreas was removed for measurement of mRNA expression. Then blood was collected through cardiac puncture (~400 µl) and placed on ice until serum separation. After that, the remaining part of the pancreas was rapidly excised and was cleaned from fat and lymph nodes on ice, then cut into pieces. A small piece of the pancreatic tissue was fixed in 8% neutral formaldehyde solution for histological analysis, the rest was immediately frozen in liquid nitrogen and stored at −80 °C until biochemical assay. For dry-wet mass measurements, a small sample was stored in an Eppendorf tube. For immunohistochemical staining procedures a small piece of the pancreas was frozen in cryomatrix at –80 °C. Blood samples were centrifuged at 2500 g for 15 min at 4 °C and the sera were stored at –20 °C until amylase activity measurement. In case of the NaTc-model, pancreata were stored only for histological analysis due to the heterogeneity of AP induction.

V.1.5. Histological examination

Formalin-fixed and paraffin embedded pancreatic tissues were sectioned to 3 µm. These sections were stained with hematoxylin and eosin (H&E) and were scored by independent experts blinded to the experimental protocol, then the scores related to the same samples were averaged. To quantify cellular damage, leukocyte infiltration and oedema grades a semiquantitative scoring system was used according to Kui et al. (2015). Briefly, oedema was scored from 0-3 points (0: none; 1: patchy interlobular; 2: diffuse interlobular; 3: diffuse

interlobular and intra-acinar), leukocyte infiltration from 0-4 points (0: none; 1: patchy interlobular; 2: moderate diffuse interlobular; 3: mild diffuse interlobular; 4: diffuse interlobular and intra-acinar). Percentage of cellular damage was also evaluated. In addition, the extent of vacuolization was determined (0: none; 1: diffuse/mild; 2: diffuse/moderate; 3: diffuse/severe).

V.1.6. Laboratory measurements

Pancreatic myeloperoxidase (MPO) activity is a hallmark of leukocytic infiltration and was measured according to Kuebler et al. (1996). MPO activities were normalized to total protein content as measured by the Lowry method (Lowry et al., 1959). To evaluate pancreatic water content, the wet weight (WW) of the pancreata was measured, then the tissues were dried for 48 h at 100 °C and the dry weight (DW) was also measured. The tissue water content was calculated as: [(WW-DW)/WW]×100. Serum amylase activity was measured on a Fluorostar Optima plate reader from BMG Labtech (Ortenberg, Germany) with a colorimetric kinetic method using a commercial amylase activity kit. Absorbance of the samples was detected at 405 nm.

V.1.7. mRNA extraction and reverse transcription

A small piece of pancreas was placed on ice in 1 ml TRIzol reagent in a 13 ml centrifuge tube and was homogenised immediately with IKA Ultra Turrax (Type: TP18/10). Then the tissue homogenate was instantly placed on liquid nitrogen and stored at –80 °C until use (for max. of 1 or 2 days). Total RNA purification was performed in three steps. In the first step, phase separation was performed by adding 200 µl of chloroform to the samples and shaking vigorously for 15 min, allowing to stand, and then centrifuging at 12000 g for 15 min at 4 °C. From the resulting 3 phases, the top aqueous phase was aspirated into an empty Eppendorf tube and 500 µl of isopropanol was added. This was vortexed and then allowed to stand for a few min and after that it was centrifuged at 12000 g for 10 min at 4 °C. RNA precipitated in the Eppendorf tubes. The supernatant was removed and 1 mL of 75% alcohol was added. It was vortexed and centrifuged at 7500 g for 5 min at 4 °C. After removal of the supernatant, the excess ethanol was evaporated briefly and then the RNA was redissolved in 70 µl of RNAse-free water. RNA was stored at –80 °C until further use.

RNA concentration was measured using a NanoDrop instrument from ThermoFisher Scientific. We considered the optimal ranges for RNA: A260/A280: 1.9-2.1 and A260/A230: 1.8-2.5. RNA integrity was examined after agarose gel electrophoresis.

2 µg of total RNA was used for reverse transcription. PCR protocol for the reverse transcription was started at 25 °C for 10 min, followed by 37 °C for 2 h, 85 °C for 5 min, then 4 °C. cDNA was stored at –20 °C until further use.

V.1.8. Real-time PCR

The total reaction mix volume was 10 µl. The components of the reaction mixture were the following: 1.67 µl cDNA sample, 0.4 mM forward and reverse primer, Luminaris Color HiGreen qPCR Master Mix and nuclease-free water. The protocol for the quantitative real-time PCR was started at 50 °C for 2 min, then 95 °C for 10 min, followed by 40 cycles of amplification: 95 °C for 15 sec, 60 °C or 64 °C for 30 sec (depending on the primers), 72 °C for 30 sec then a melt curve from 70 °C to 95 °C for 5 sec with 0.5 °C increment. Our primers used in relative gene expression measurements (Table 2.) were checked with the Oligoanalyzer program from Integrated DNA Technologies (Iowa, USA; https://www.idtdna.com/pages/tools/oligoanalyzer). Gradient PCR was and performed to determine the appropriate annealing temperature of the primers. The *Slc26a3* and *Slc26a6* primer pairs have been used previously in our laboratory (Molnár et al., 2020). The housekeeping gene in mRNA studies was *Rplp0*.

Gene	5'-3' Primer pairs	Product (bp)
Cftr	F: GACGAGCCAAAAGCATTGAC	157
	R: TGGTCCAGCTGAAGAAGAGT	
Slc26a3	F: CTCGGACCCCAATGCTTCTT	127
	R: CCCCAGGAGCAACTGAATGA	
Slc26a6	F: GAGCTGTTTGCAACGCTTGT	121
	R: CCTGGTTACTGTCCACACGG	
Ck19	F: ATCGTCTCGCCTCCTACTT	250
	R: TCTGTCTCAAACTTGGTTCTG	
Rplp0	F: AGATTCGGGATATGCTGTTGGC	109
	R: TCGGGTCCTAGACCAGTGTTC	

Table 2. Oligonucleotide primer pairs used for determination of relative gene expression. CK19, cytokeratin-19; Rplp0, ribosomal protein lateral stalk subunit P0.

V.1.9. Immunohistochemistry

The cryomatrix-embedded pancreatic tissues derived from animals were sliced by a Leica Cryostat at 7 µm thickness. Sections were fixed in 2 % paraformaldehyde for 20 min. Between each step, tissue slices were washed three times with Tris-buffered saline (TBS) solution. Antigen retrieval was performed with 0.1 % Triton X-100 in TBS solution for 10 min. Blocking was performed for 1 h with 5 % bovine serum albumin-TBS solution. These sections were then incubated with anti-CFTR rabbit polyclonal antibody (dilution 1:400) overnight at 4 °C. The following day the samples were incubated with AlexaFluor 488 goat anti-rabbit secondary

antibody (dilution 1:500) for 1 h in the dark at room temperature. After a few washing steps, co-immunostaining was performed with the AlexaFluor647-conjugated cytokeratin-19 (CK19) antibody (dilution 1:100, CK19 is a ductal marker protein). Nuclei were counterstained with Hoechst 33342 (dilution 1:400). Tissue slices were mounted with Fluoromount and then analysed using a Zeiss LSM 880 confocal laser scanning microscope (Carl Zeiss Technika Kft., Budapest, Hungary). To quantify pancreatic ductal CFTR and CK19 expression, three or four representative large tile scan images were taken from each group by with the confocal microscope (in average 1500 x 1000 μm). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to convert images to grey scale (16 bit), and threshold function was used to select the positively stained area based on the fluorescence intensities. The tissue-free areas were excluded from the calculation.

V.1.10. Fluid secretion and intracellular pH measurements in cultured pancreatic ducts

Intra-/interlobular pancreatic ducts were isolated from control and AP mice after collagenase digestion by microdissection as described previously (Argent et al., 1986). In case of fluid secretion measurements, ducts were cultured for 6-14 h (which allowed sealing of their open ends) at 37 °C in a humidified atmosphere containing 5% CO_2. Some ducts were treated with 3 μM VX-661 and 1 μM VX-770 during incubation, while others subjected to the vehicle (0.5% DMSO) or only the medium.

Fluid secretion into the closed luminal space of the cultured pancreatic ducts was analysed using a swelling method (Fernandez-Salazar et al., 2004; Pallagi et al., 2014). Then ducts were transferred to a perfusion chamber and were attached to a coverslip pre-coated with 0.05 mg/ml poly-L-lysine. The ducts were perfused with different solutions in the following order: 1) standard HEPES, 2) standard HEPES with 5 μM forskolin 3) standard HCO_3^-/CO_2 with 5 μM forskolin. Bright-field images were acquired at 1-min intervals using a Zeiss Axio Observer 7 with CMOS camera (Orca Flash 4.0 LT, Hamamatsu Photonics, Hamamatsu City, Japan). The integrity of the duct wall was checked at the end of each experiment by perfusing the chamber with a hypotonic solution (standard HEPES-buffered solution diluted 1:1 with distilled water). Digital images of the ducts were analysed using ImageJ software to obtain values for the area corresponding to the luminal space in each image.

pH_i measurements were started immediately after isolation and were carried out within 8 h thereafter using IX71 live cell imaging fluorescence microscope and CellR imaging system from Olympus (Budapest, Hungary). Alkali load method was applied to measure pancreatic ductal HCO_3^- secretion (Venglovecz et al., 2008). The HCO_3^- secretion was estimated by the rate of pH_i recovery from alkalization. The isolated ducts were loaded with the pH sensitive

BCECF-AM fluorescent dye (2 μM) for 20-30 min in standard HEPES solution at 37 °C in a humidified atmosphere containing 5 % CO_2. After that, ducts were perfused with solutions in the following order: 1) standard HEPES, 2) standard HCO_3^-/CO_2, 3) NH_4Cl in HCO_3^-/CO_2, 4) standard HCO_3^-/CO_2, 5) standard HEPES. Exposing ducts to 20 mM NH_4Cl caused alkalization of pH_i. The perfusion rate was 4-6 ml/min. Four to ten small areas (region of interests, ROIs) of 5-10 cells in each intact duct were monitored. The ducts were excited with light at wavelengths of 490 and 440 nm, and the 490/440 fluorescence emission ratio was measured at 535 nm. One pH_i measurement was obtained per second. The extent of pH_i change ($\Delta pH/\Delta t$) was calculated by linear regression analysis.

V.1.11. Statistical analysis

Graphs were generated by GraphPad Prism 9.2.0 (GraphPad Software, San Diego, CA, USA) or by Microsoft Excel and PowerPoint (Redmond, WA, USA). Data are presented as means ± SD in the case of Cer-AP and as means ± SEM in NaTc-AP rats. Experiments were evaluated by one- or two-way ANOVA followed by the Tukey HSD post hoc test in Cer-AP or Holm–Sidak post hoc tests in NaTc-AP (SPSS, IBM, Armonk, NY, USA). $P<0.05$ was accepted as statistically significant.

V.2. Systematic review and meta-analysis

V.2.1. Registration and PICO

Our systematic review and meta-analysis was conducted according to the protocol previously registered in the PROSPERO database (https://www.crd.york.ac.uk/PROSPERO/, ID: CRD42017071264). The methodology for this analysis followed recommendations by Stroup et al. (2000) and the guidelines for the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (Shamseer et al., 2015). The analysis was based on the Problem, Intervention, Comparison intervention, and Outcome (PICO) model (Shamseer et al., 2015). The problem was AP. The intervention was HTG with various groups formed for the analysis: >1.7, 1.7–5.6, 1.7–11.3, >5.6 and >11.3 mM seTG. The comparison interventions were normal (<1.7), <5.6, 1.7–5.6, 1.7–11.3 and <11.3mM seTG. Different outcomes were investigated: AP severity, mortality, pancreatic necrosis, persistent organ failure (POF) and multi-organ failure (MOF), pulmonary and renal failure, and admission to an intensive care unit (ICU).

V.2.2. Article search strategy

The search was carried out in late August 2017. Observational prospective and retrospective cohorts, and case control studies were identified in Embase (published from 1948 to July 2017) and PubMed Library (published from 1961 to July 2017). Furthermore,

ClinicalTrials.gov was also screened for additional unpublished data. The search contained the following terms for Embase: pancreatitis AND ('triglyceride'/exp OR triglyceride OR hypertriglyceridemia OR 'hyperlipidemia'/exp OR 'hyperlipidemia') AND [english]/ lim AND ('human'/de OR patient OR patients) NOT ('conference abstract'/it OR 'review'/it OR 'case report'/de OR 'nonhuman'/de OR 'practice guideline'/de). The following terms were used for PubMed: pancreatitis AND (hyperlipidaemia OR hyperlipidemia OR triglycerides OR triglyceride OR hypertriglyceridaemia OR hypertriglyceridemia) AND (human OR patient OR patients) AND English NOT "case reports" [Publication Type]. The search terms for Clinicaltrials.gov were pancreatitis and hypertriglyceridemia.

V.2.3. Eligibility criteria

Articles were included if they fulfilled the following criteria:

1. Case control or cohort studies.

2. Studies involving AP patients.

3. HTG (>1.7mM) was present in at least one of the groups under investigation

4. SeTGs were defined.

5. Outcome data were provided for at least one of the following: severity of AP according to the Revised Atlanta Classification, mortality, pancreatic necrosis, POF, MOF, pulmonary failure, renal failure, and intensive care unit (ICU) admission.

6. Written in English.

The seTG in different groups used as controls was below 1.7, 5.6 or 11.3 mM as well as within the 1.7–5.6 and 1.7–11.3 mM ranges. Systematic reviews, meta-analyses, reviews, conference abstracts, letters, replies, reports, commentaries, notes, case studies, animal studies, practice guidelines and non-English-language (e.g. Chinese-, Polish- and German-language) articles were excluded from the analysis.

V.2.4. Study selection and data extraction

Relevant studies were manually screened by two independent authors. In the first step, publications were screened by title and abstract, then the potentially eligible studies were independently assessed for eligibility criteria by the same two review authors. The investigators after that extracted the characteristics of proper studies and also the data, which was then statistically analysed. Discrepancies were resolved by discussion with other two authors.

V.2.5. Quality assessment of the articles

The Newcastle–Ottawa Scale (NOS) was used to assess the quality of the articles included (Stang, 2010; Wells et al., 2017). Since seTG decreases rapidly when food intake is restricted (Dominguez-Muñoz et al., 1991), the NOS was supplemented with another scoring system in

which the articles were also evaluated based on the timing of the seTG measurement. The detailed evaluation method can be found in the supplementary part of the original article.

V.2.6. Data analyses

The statistical analysis was performed with Stata 11 SE (StataCorp LLC, College Station, TX, USA). The numbers of patients with regard to AP severity, mortality, pancreatic necrosis, POF, MOF, pulmonary failure, renal failure and ICU admission were used to calculate odds ratios (OR) to compare these outcomes in different seTG groups. ORs were pooled using the random effects model with the DerSimonian–Laird estimation and displayed on forest plots. Summary OR estimation, p value and 95% confidence interval (CI) were calculated. ORs with corresponding CIs and p values are indicated in the text according to the following order: OR [CI; p]. $P<0.05$ was considered a significant difference from summary OR=1. The different ORs were compared using the analysis of variance with random effect weights.

Heterogeneity and publication bias: Statistical heterogeneity was analysed using the I^2 statistic and the chi-square test to acquire probability values; $p<0.05$ was defined to indicate significant heterogeneity. The small-study effect was visually investigated on funnel plots.

VI. RESULTS

VI.1. Animal experiments

VI.1.1. The time course of cerulein-induced acute pancreatitis severity

The progression of Cer-AP was followed from 0 to 72 h. Representative histological images show pancreatic tissues of mice sacrificed at different time points (Figure 6A). Cer injection caused the greatest degree of cell damage at 24 h (Figure 6A-B). This is adequately supported by the scoring results of vacuolization (Figure 6C). Cell damage and vacuolization were significantly decreased at 48 and 72 h compared to 24 h groups. Leukocyte infiltration was significantly increased at 6, 12, 24, and 72 h after the first Cer injection compared to control (Figure 6D). Changes in pancreatic MPO activity closely followed leukocyte infiltration and showed marked increases at 12 and 24 h (Figure 6E). AP evoked significant elevations in pancreatic water content and serum amylase activity at 12 and 24 h compared to the control (0 h) group (Figure 6F-G). Serum amylase activity was highest at 12 h, which then decreased back to control levels after 48 h (Figure 6G). At 48-72 h, almost all histological and laboratory parameters showed decreased tendency compared to the 12 or 24 h groups.

VI.1.2. Changes in CFTR expression and staining morphology during acute pancreatitis

Figure 7 shows the mRNA expressions and protein staining during the time course of AP. The expression of *Cftr* mRNA was markedly increased during AP from 24 h compared to the control (Figure 7A). The peak was detected at 48 h and almost 20-fold increase was measured in *Cftr* mRNA amount compared to the heathy group. This tendency was also observed in case of the ductal marker *Ck19* (Figure 7B), but its mRNA had less marked increased expression than *Cftr*. *Ck19* mRNA expression was highest at 12 and 24 h after the initiation of AP. *Slc26a3* mRNA expression was also increased at 24 h, while the mRNA of *Slc26a6* was decreased between 24 and 72 h (Figure 7C-D).

The percentage of area staining of CFTR and CK19 proteins was determined by fluorescent immunostainings. Figure 7E shows representative images which was used to calculation of area staining. Six percent of pancreatic tissue in untreated group stained for CFTR (Figure 7F). At the beginning of AP (6 and 12 h), the CFTR staining area showed decrease in tendency, but at 24 h the protein expression significantly increased compared to the control or 6-12 h treatment groups. The increase of CFTR protein expression is in accordance with the results of the mRNA measurements. CK19 staining area in the control group was approximately 20 % (Figure 7G). This area of CK19 decreased in pancreatic tissue at the beginning of AP (6 h), while later on CK19 expression did not differ from the control group.

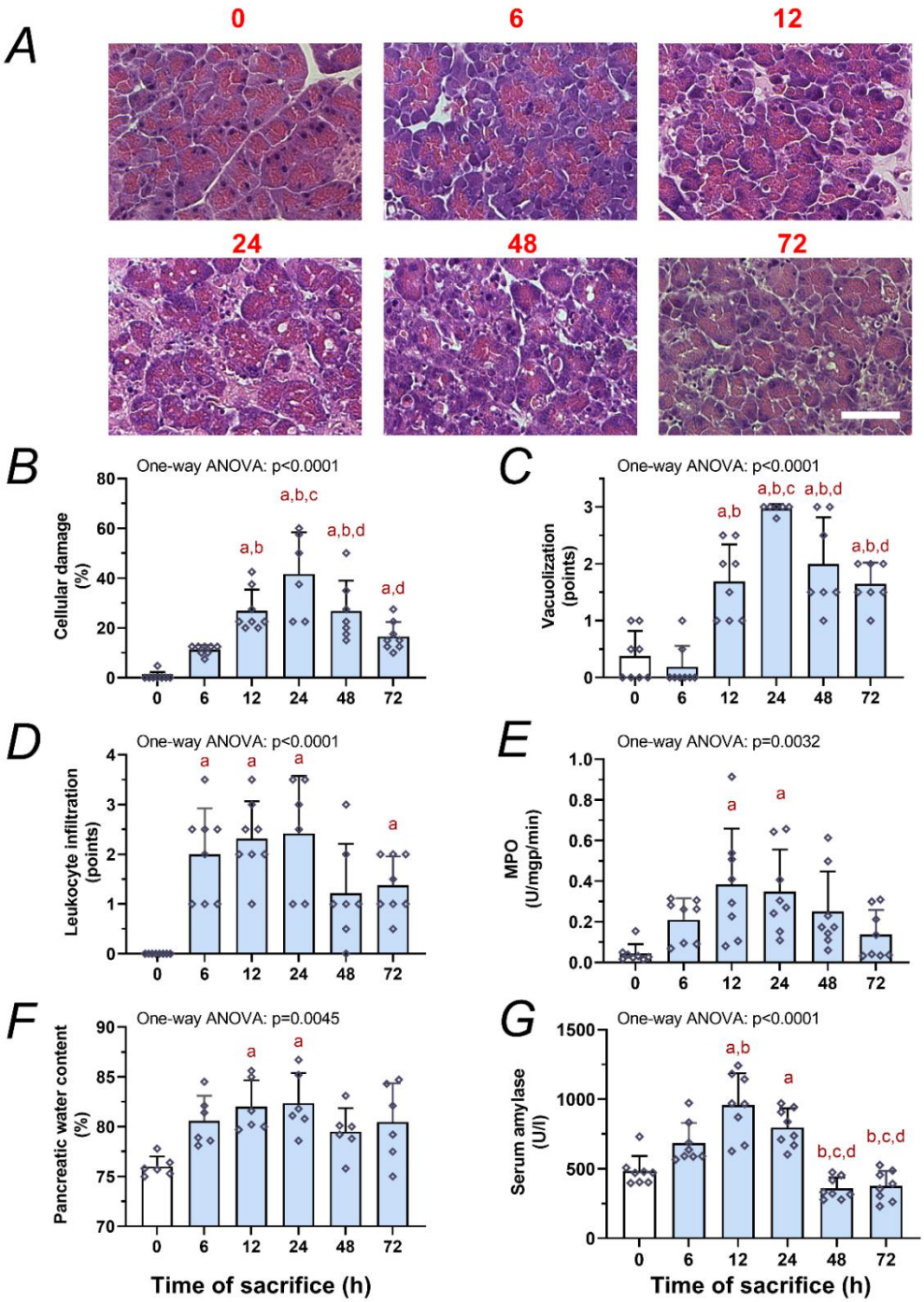

Figure 6. Disease severity course in Cer-AP. A, representative hematoxilin and eosin (H&E) histopathological images of pancreatic tissues from mice. The groups were established based on time of sacrifice after AP induction (first Cer injection): 0, 6, 12, 24, 48, 72 h. Scale bar represents 100 µm. Bar charts show the extent of pancreatic B, cellular damage; C, vacuolization; D, leukocyte infiltration; E, myeloperoxidase (MPO) activity; F, water content; and G, serum amylase activity. Values represent means with standard deviation, n=6-8. One-way ANOVA was performed followed by Tukey HSD post-hoc test. Statistically significant differences ($p<0.05$) were detected and marked with: (a) vs. 0h; (b) vs. 6h; (c) vs. 12h; (d) vs. 24h.

Detailed ductal structures were also captured after CFTR and CK19 immunostaining (Figure 8). The physiological location of CFTR is in the apical membrane of the pancreatic ducts. Clearly, detectable ductal morphology was observed in the control animals in cases of both CFTR and CK19. The lumens of the stained intralobular ducts were approximately 2-3 µm in diameter, which could be followed through several µm. The nuclei were located close to the

ductal lumen. Bigger interlobular ducts did not stain for CFTR. Notably, AP even from 6 h disturbed the characteristic structure of the ductal tree. CFTR was mislocalized and both CFTR and CK19 proteins showed diffuse and perinuclear appearance. At 72 h after the initiation of the disease, some duct like structures appeared in stained tissue slices (Figure 8, yellow arrow). CK19 and CFTR showed similar staining morphologies at all time points.

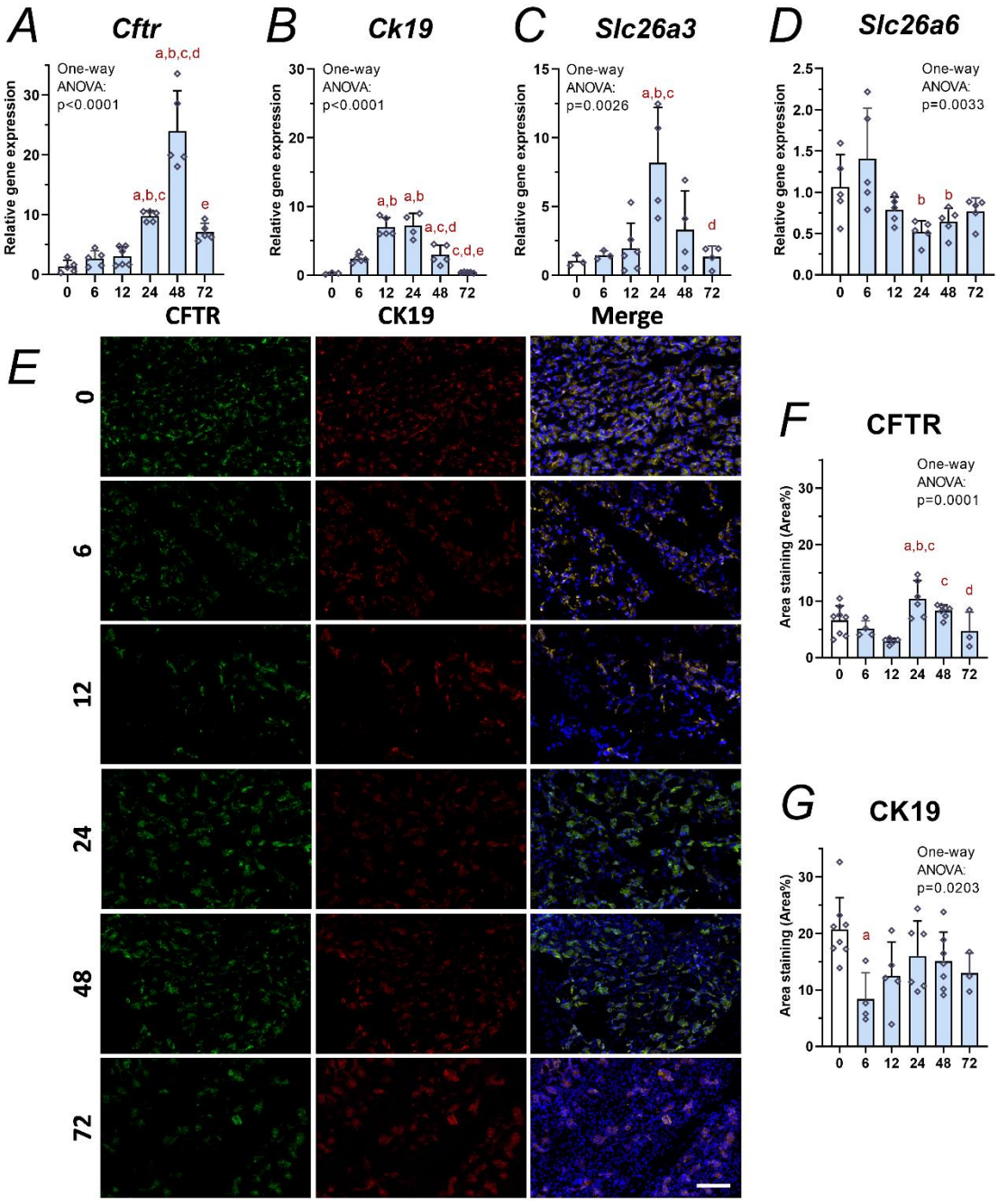

Figure 7. Relative gene expression of *Cftr, Ck19, Slc26a3, Slc26a6* and immunostainings of CFTR and CK19 proteins during the course of AP. Bar charts show the relative gene expression of A, *Cftr*; B, *Ck19*; C, *Slc26a3*; and D, *Slc26a6*. Representative immunofluorescent or fluorescent images (CFTR, CK19, and cellular nuclei stainings) of pancreatic tissues from control and Cer-treated animals. Scale bar is 100 µm. Bar charts show the staining area of F, CFTR and G, CK19 proteins. Values represent means with standard deviation, n=3-7. One-way ANOVA was performed followed by Tukey HSD post-hoc test. Statistically significant differences ($p<0.05$) were detected and marked with: (a) vs. 0h; (b) vs. 6h; (c) vs. 12h; (d) vs. 24h; (e) vs. 48h.

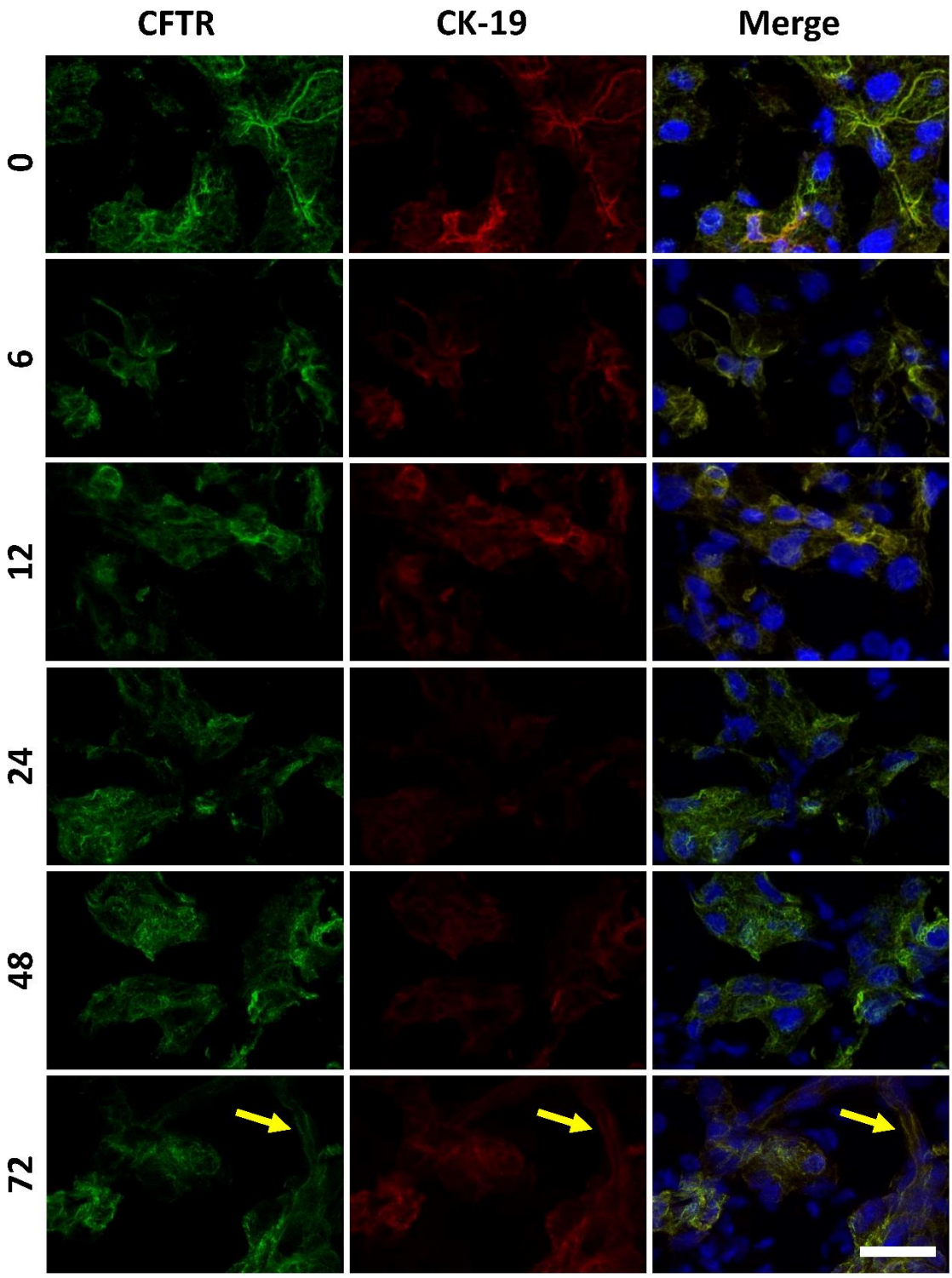

Figure 8. The pancreatic ductal morphology during AP. Fluorescent immunostaining of CFTR (green), CK19 (red) proteins and cellular nuclei (blue) in pancreatic tissue of mice with AP observed at 0, 6, 12, 24, 48, 72 h after AP induction. Duct like structures appeared in stained tissue slices (yellow arrow). Scale bar = 20 μm.

VI.1.3. Pancreatic ductal HCO_3^- secretion during the course of acute pancreatitis

Alkali load method and measurement of pH_i changes during the cellular regeneration phase provided estimation of the HCO_3^- secretory function of isolated pancreatic ducts. This is mainly carried

out by apical transporters (CFTR, SLC26A3, SLC26A6). When cellular alkalosis was stopped by ammonium elimination, the cells shortly became acidotic. The cellular regeneration from acidosis can activate basolateral transporters (e.g. NBC, NHE or H^+-ATPase). Therefore, the regeneration rate from acidosis refers to the activity of basolateral transporters. At the early phase (6 h) of AP, HCO_3^- secretion by apical transporters was significantly increased as demonstrated by regeneration from alkali load (Figure 9A; 0 h vs. 6 h). Basolateral transporter activity was also significantly elevated when regeneration from acidosis was measured (Figure 9B; 0 h vs. 6 h). However, from 12 to 72 h, the response to alkalosis and acidosis were not significantly different vs. the control group.

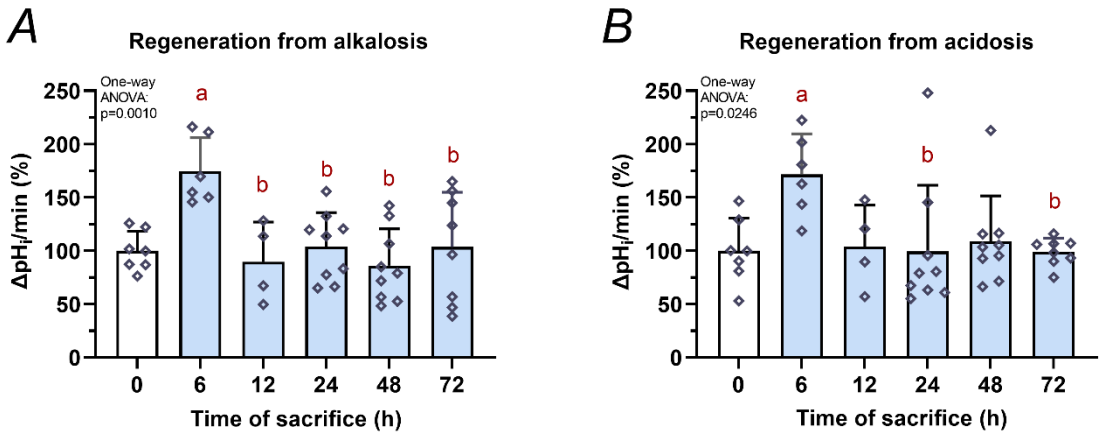

Figure 9. Ductal HCO_3^- secretion during the course of AP. Intra-/interlobular pancreatic ducts were isolated from control (0 h) and AP mice 6-72 h after the first Cer injection. The measurements of intracellular pH changes (ΔpH_i) are plotted for regeneration from A, alkalosis and B, acidosis. Values represent means with standard deviation, n=4-9 ducts. One-way ANOVA was performed followed by Tukey HSD post-hoc test. Statistically significant differences ($p<0.05$) were detected and marked with: (a) vs. 0h; (b) vs. 6h.

VI.1.4. The combination of CFTR corrector VX-661 and CFTR potentiator VX-770 decreased the severity of acute pancreatitis

The combination of VX-661 and VX-770 by itself did not induce any gross adverse effects. In fact, the morphology of the pancreas was normal after administration of VX-661+VX-770, and the histological and laboratory parameters were also similar to the non-treated group (data are not shown in this thesis, but can be found in the respective publication, Fűr et al., 2021). The effects of VX-661+VX-770 on AP are shown in Figure 10. Representative histological sections showed that AP damaged the pancreatic tissue and VX-661+VX-770 combination could ameliorate this damage (Figure 10A). Almost all measured parameters were increased in AP groups compared to the control group (Figure 10B-F). VX-661+VX-770 pre-treatment significantly decreased AP severity based on the extent of cell damage (Figure 10B). We could not observe any significant difference in vacuolization over time or treatment in the AP groups (Figure 10C). Measurements of pancreatic leukocyte infiltration and MPO activity showed similar kinetics, with no significant difference in the AP groups (Figure 10D-E). No change was observed in pancreatic water content when the AP group was compared with the AP+VX-661+VX-770 group (Figure 10F). In case of serum amylase activity, no significant difference was measured between the control and treated groups (Figure 10G).

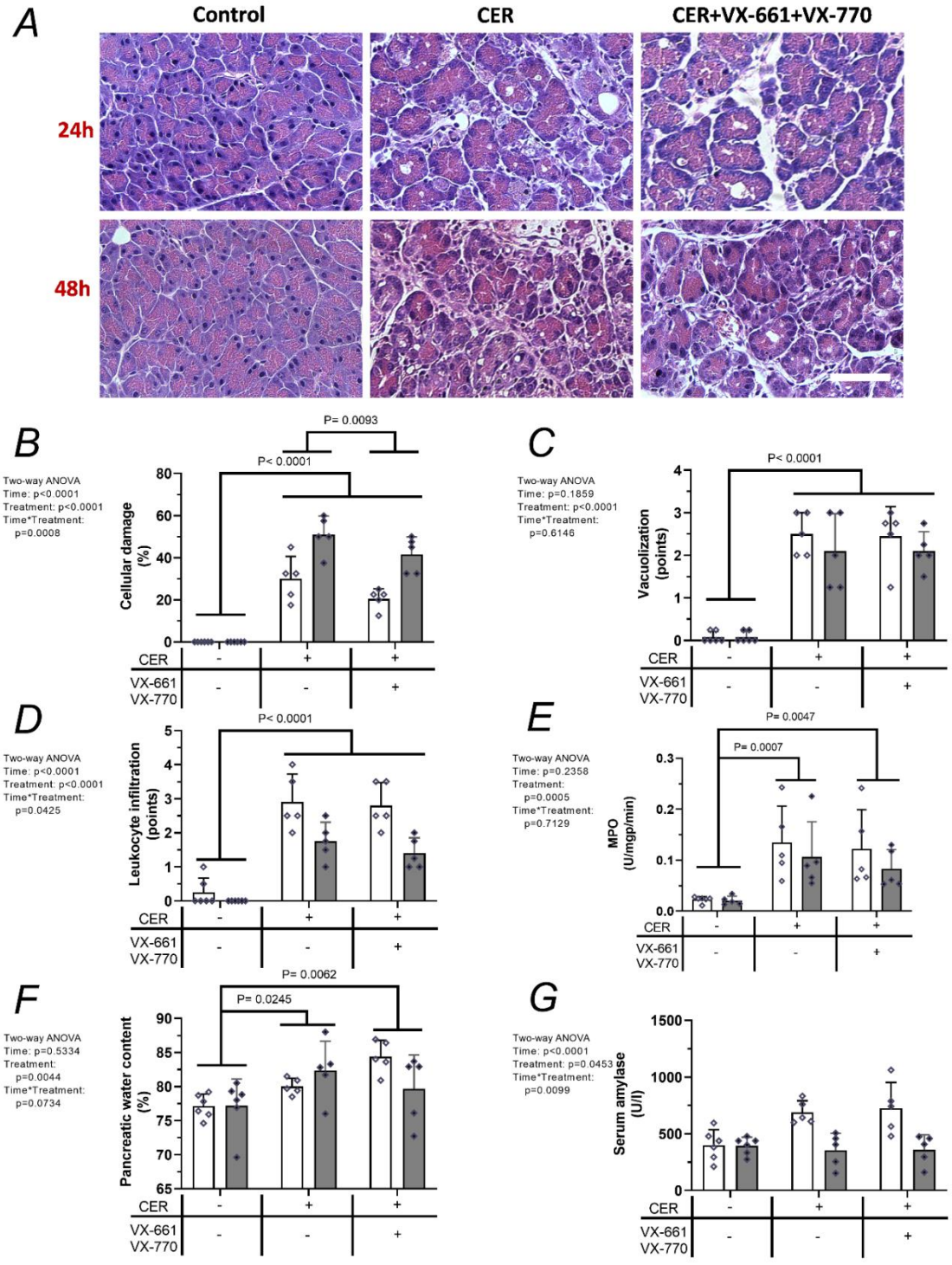

Figure 10. The effect of CFTR corrector VX-661 and CFTR potentiator VX-770 on the severity of AP. A, representative histopathological images of pancreatic tissues of the treatment groups at 24 or 48 h termination. Scale bar represents 100 µm. Bar charts show the extent of pancreatic B, cellular damage; C, vacuolization; D, leukocyte infiltration; E, MPO activity; F, water content; and G, serum amylase activity measurements. Light and dark grey bars show 24 and 48 h measurements, respectively. Values represent means with standard deviation, n=5-6. Two-way ANOVA was performed followed by Tukey HSD post-hoc test.

VI.1.5. The effect of CFTR corrector VX-661 and CFTR potentiator VX-770 on ductal morphology and protein expression in acute pancreatitis

CFTR and CK19 co-immunostainings showed normal ductal structures at 24 and 48 h in control groups (Figure 11). AP disturbed the staining morphology of CFTR and CK19 at both 24 and 48 h. VX-661+VX-770 pre-treatment could not restore or improve the damaged ductal structure as demonstrated by CFTR or CK19 staining (Figures 11 and 12). CFTR protein expression was increased by AP while CK19 expression was unchanged (Figure 12B-C). VX-661+VX-770 pre-treatment had no effect on AP-induced alterations of CFTR and CK19 protein expressions.

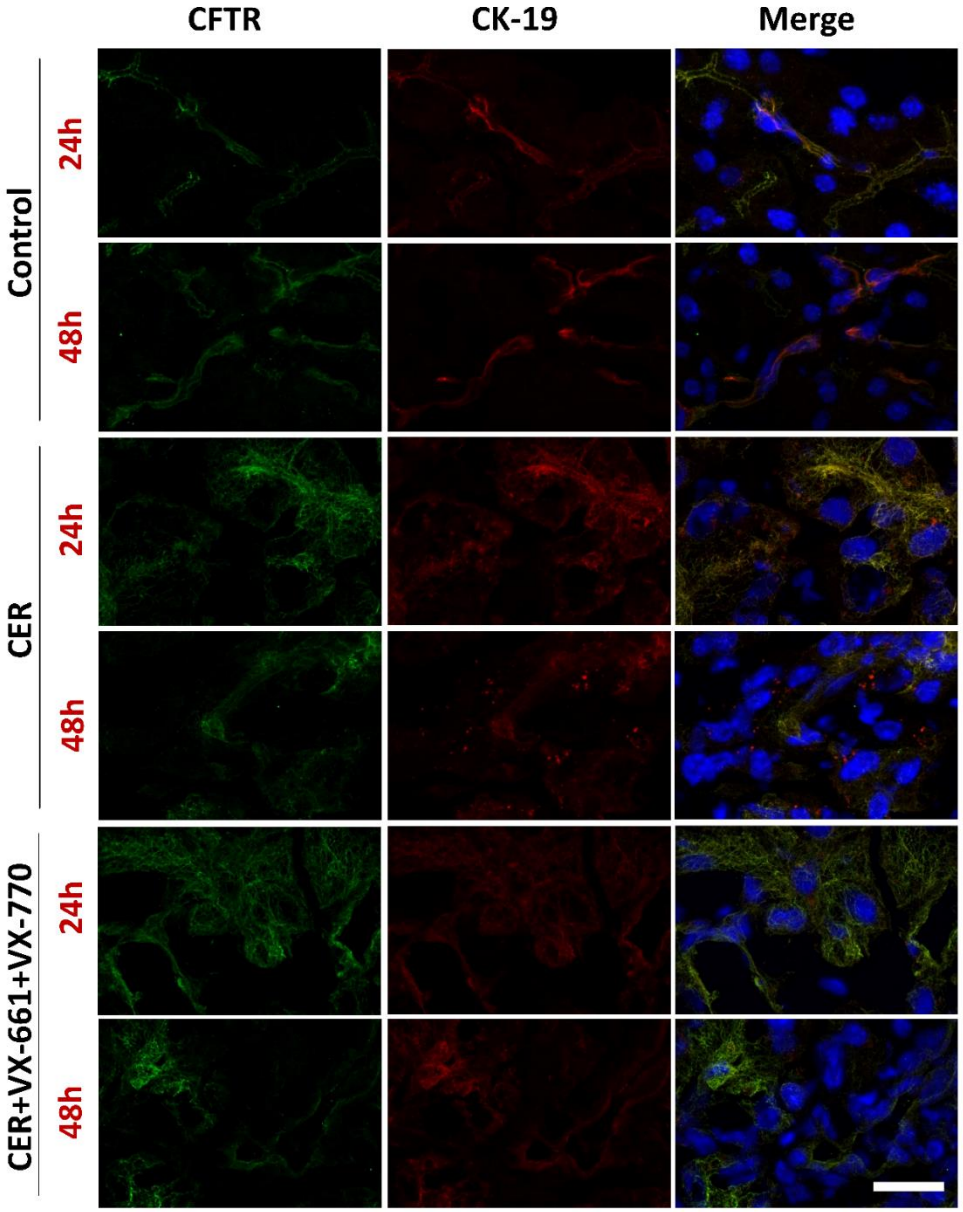

Figure 11. The effect of VX-661 and VX-770 on ductal morphology during AP. Immunofluorescent staining of CFTR (green) and CK19 (red) proteins and cellular nuclei (blue) in pancreatic tissues from mice at 24 or 48 h after AP induction. Treatment groups: control; Cer-AP; Cer-AP+VX-661+VX-770. Scale bar = 20 μm.

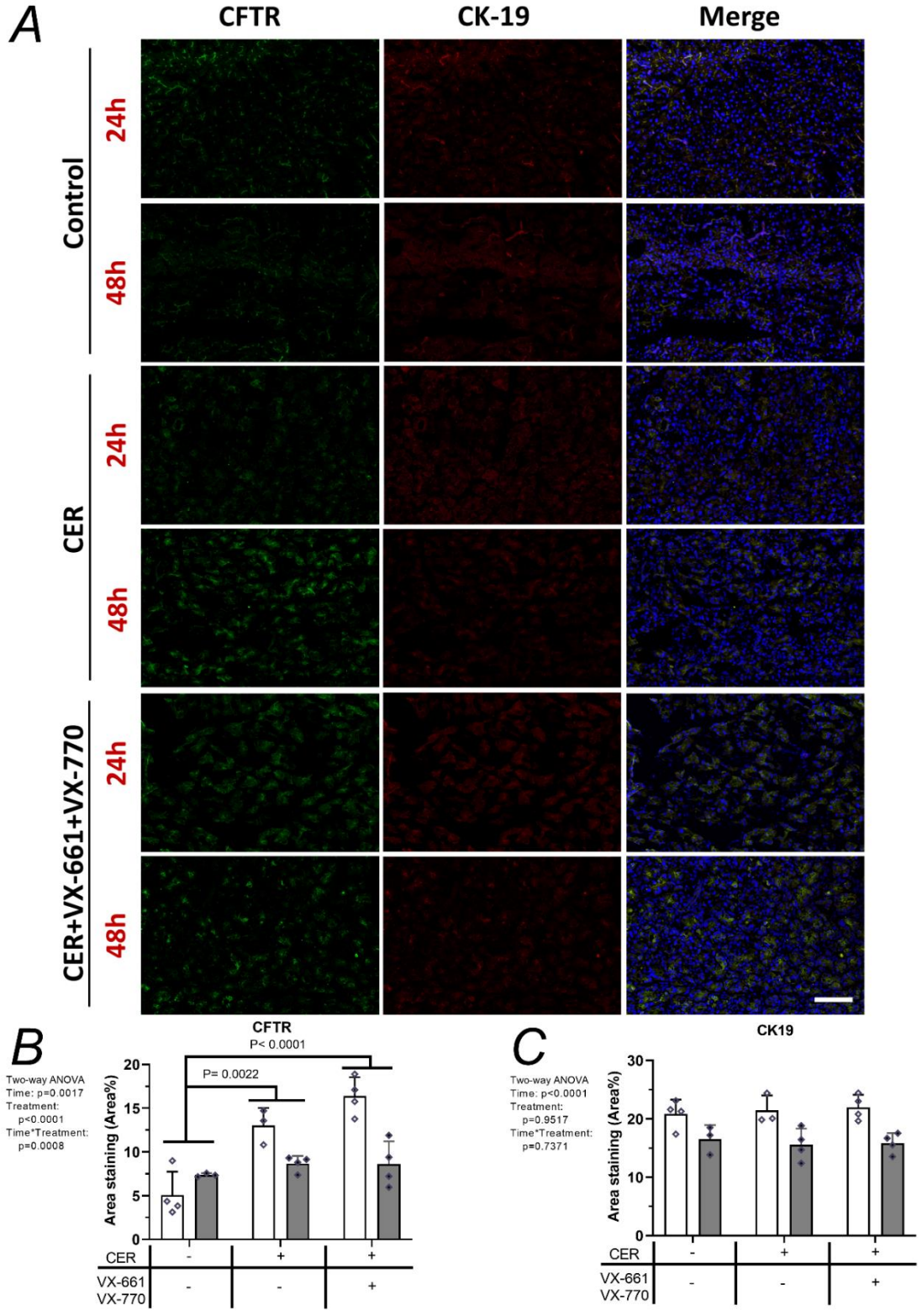

Figure 12. The effect of VX-661 and VX-770 on the protein expression of CFTR and CK19 during AP. A, representative immunofluorescent images (CFTR, CK19, and cellular nuclei stainings) of pancreatic tissues. Treatment groups: control; Cer-AP; Cer-AP+VX-661+VX-770. Bar charts show the staining area of B, CFTR and C, CK19 proteins. Scale bar is 100 μm. Light and dark grey bars show 24 and 48 h measurements, respectively. Values represent means with standard deviation, n=3-5. Two-way ANOVA was performed followed by Tukey HSD post-hoc test.

VI.1.6. VX-661 and VX-770 enhance fluid secretion in isolated pancreatic ducts from mice with acute pancreatitis

To investigate if fluid secretion is influenced by the treatment with VX-661 and VX-770, isolated ducts (treated with or without 0.5 % DMSO/VX-661 and VX-770) from control and AP mice were used, and their swelling was followed (Figure 13). The cAMP agonist forskolin treatment significantly enhanced the swelling of ducts from control animals, especially in HCO_3^-/CO_2 containing buffer. DMSO administration did not influence changes in relative ductal luminal volume. Therefore, the corresponding groups treated with or without DMSO were combined (i.e., Cer and Cer+DMSO). Ducts isolated from Cer-treated animals showed tendency towards increased swelling rates compared to the PS-treated control mice, but this did not reach statistical significance (Figure 13). Interestingly, VX-661 and VX-770 treated ducts showed significantly increased fluid secretory rate compared to the non-VX treated ducts derived from AP animals (Figure 13B).

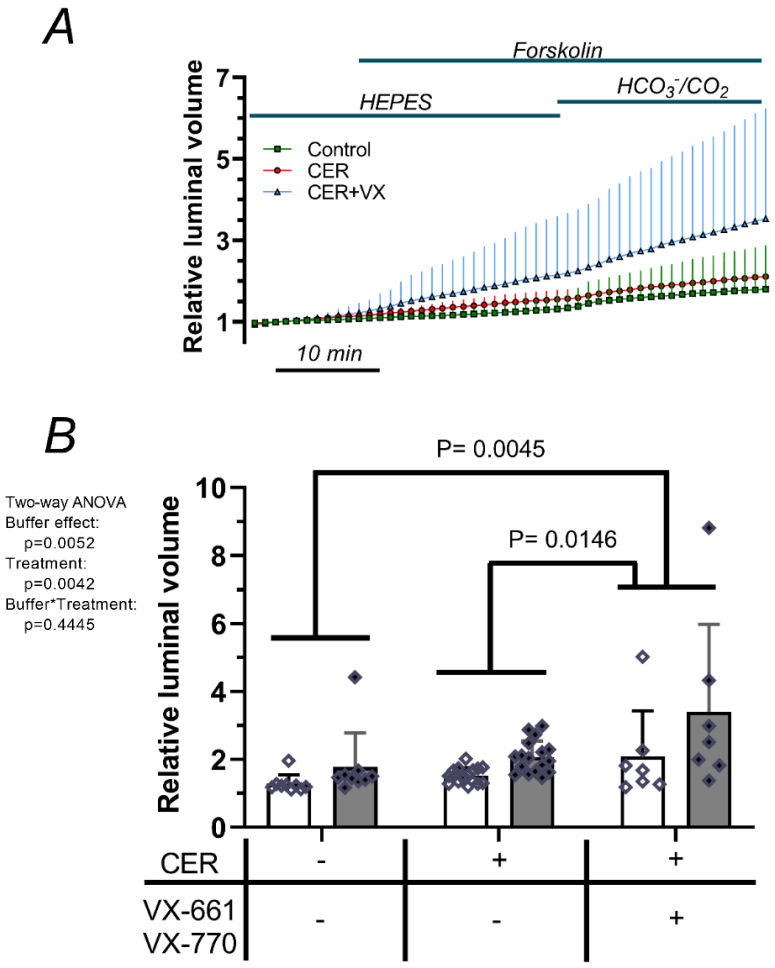

Figure 13. The effect of VX-661 and VX-770 on the fluid secretion of isolated pancreatic ducts. Intra-/interlobular pancreatic ducts were isolated from control and AP mice 6 h after the first Cer injection. Thereafter, ducts were cultured for 6-14 h (which allowed sealing of their open ends) at 37 °C in a humidified atmosphere containing 5% CO_2. 3 µM VX-661 and 1 µM VX-770 or their vehicle (DMSO) were administered in the culture media of some AP ducts. Since DMSO had no effect on the swelling of ducts isolated from AP mice, the corresponding groups treated with or without DMSO (media) were combined. A, the line diagram shows changes in relative luminal volume of pancreatic ducts derived from control and AP mice with or without VX treatment in response to administration of standard HEPES and HCO_3^-/CO_2 containing solutions. Fluid secretion was stimulated with the cAMP agonist forskolin. B, Bar charts show the relative luminal volume measured before the end of 'standard HEPES buffer+forskolin' (light bars) and 'standard HCO_3^-/CO_2 buffer+forskolin' (dark bars) perfusion. Values represent means with standard deviation, n=7-17. Two-way ANOVA was performed followed by Tukey HSD post-hoc test.

VI.1.7. The effect of fentanyl post-treatment on acute pancreatitis

I.d. infusion of NaTc-induced AP in the head but not in the tail of the pancreas (not shown), which is in accord with the finding of others (Perides et al., 2010). Therefore, only the pancreatic heads were used for analysis. NaTc elevated the extent of pancreatic necrosis, leukocyte infiltration and oedema (Figure 14A-D). Both necrosis and immune cell infiltration were decreased by the higher dose of FE (0.2 mg/kg, Figure 4B-C), while the score of oedema did not change in the AP groups after FE treatment (Figure 14D). Serum amylase activity also decreased in the NaTc+3x0.2 mg/kg FE group versus the AP group without FE treatment (Figure 14E).

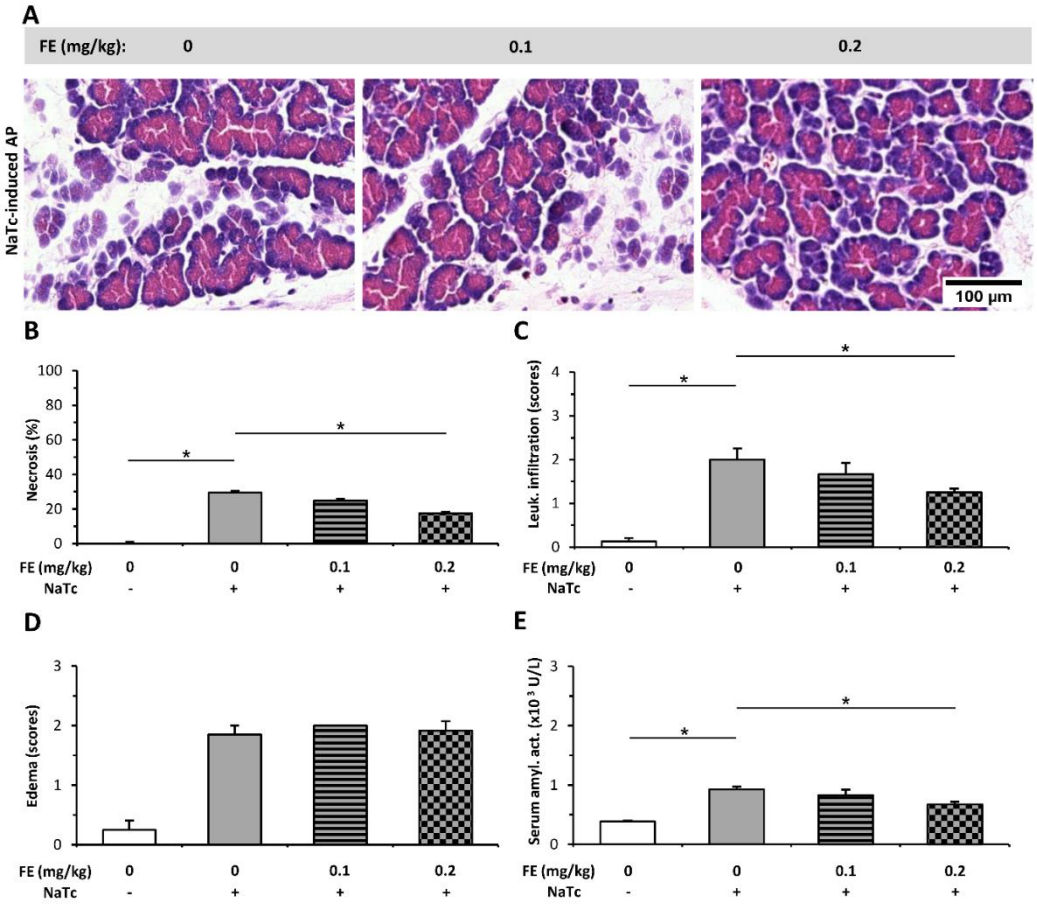

Figure 14. FE treatment started after induction of AP with NaTc reduces disease severity. A, representative histopathological images of pancreatic tissues of the treatment groups. Bar charts show the extent of pancreatic B, necrosis, C, leukocyte infiltration, D, oedema, and E, serum amylase activity measurements. Values represent mean with standard error, n=9-12. Two-way ANOVA was performed followed by Holm-Sidak post-hoc test. *: $p<0.05$.

VI.2. Meta-analysis of the effect of serum triglyceride concentration on the outcome of acute pancreatitis

VI.2.1. Study selection

The search for articles in three databases resulted in 2261 records (Figure 15). After removing duplicates and screening titles and abstracts, 90 articles were assessed in full text for eligibility. Of these manuscripts, 29 prospective and retrospective cohorts seemed to be suitable for data collection. However, in 13 publications, seTGs were defined inappropriately (e.g. <1.88 mM was identified as normal) or the outcome data could not be used. Therefore, these 13 publications were removed from the assessment, and only 16 articles were included in the statistical analysis (in which the seTG ranges or the outcome data were appropriate). These studies were published between January 2000 and March 2016.

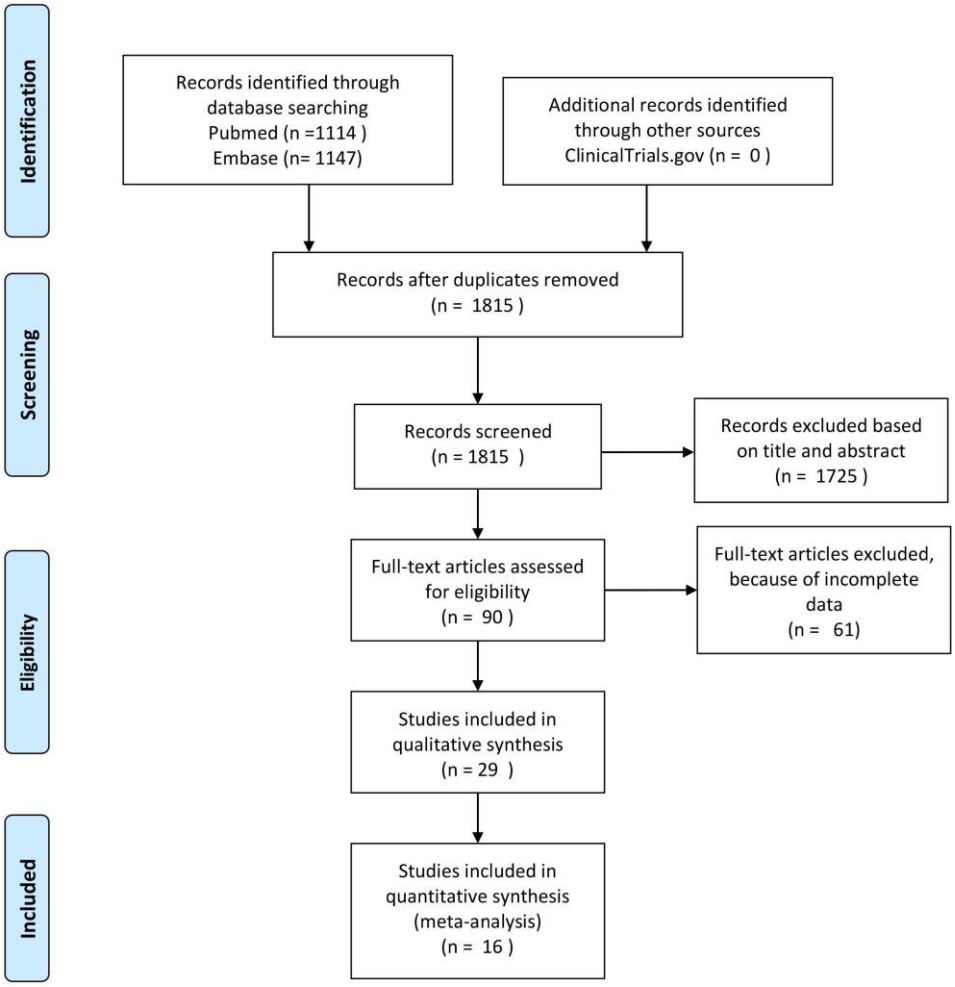

Figure 15. Flow diagram for article selection.

VI.2.2. Characteristics of studies

Both single- (13) and multicentre (3) cohort studies were included. Population sizes ranged from 43 to 3203, and six trials involved over 300 patients. The aetiology of AP was noted in all the studies, eight comprised HTG-AP (>11.3 mM seTG), and twelve contained alcoholic and biliary pancreatitis patients. Other aetiologies, such as post-ERCP, idiopathic, mixed and drug-induced AP, were also included in some articles. The studies were performed in the following countries: China (nine cohorts), the USA (three cohorts), Hungary (two cohorts), the UK (one cohort) and Spain (one cohort). Table 3 summarizes the characteristics of the cohorts involved. During the quality assessment, we evaluated patient selection, comparability of the groups, outcome data, and the timing of the seTG measurement. These quality scores are depicted in the corresponding figures.

First author, year	Source	Study design	Inclusionperiod	Centre	Patient number: used for analysis/total (n/n)	AP aetiology	Groups based on seTG (mM); patients (n)
Jiang, 2005	Chin J Dig Dis	NA	Jan 2000–Jan 2002	S	99/99	B, A, O	<1.7; 71 >1.7; 28
Balachandra, 2006	Int J Clin Pract	P	2001	S	40/43	B, A, I, pE	<1.8; 26 >1.8; 14
Deng, 2008	World J Gastroenterol	R	Mar 2003–Dec 2004	S	176/176	A, B, D, L-Asp, chemotherapy, pregnancy	<5.65; 131 ≥5.65; 45
Baranyai, 2012	Clin Lipidol	R	Jan 2007–Dec 2009	S	351/351	HTG, O	<11.3; 328 >11.3; 23
Ivanova, 2012	Hepatobiliary Pancreat Dis Int	P	Mar 2006–Feb 2007	S	133/133	B, A, I, HTG, O	<11.28; 126 >11.28; 7
Zeng, 2014	Am J Med Sci	R	N.D.	S	340/340	B, B+HTG	<1.70; 250 1.70–2.25; 18 2.26–5.64; 31 ≥5.65; 41
Nawaz, 2015	Am J Gastroenterol	P	Jun 2003–Jun 2004	S	201/201	B, A, I, HTG, O	<1.70; 115 1.70–2.26; 20 2.27–11.32; 41 ≥11.33; 25
Zheng, 2015	Pancreas	R	Jan 2006–Dec 2010	M	2461/2461	B, A, HTG, O	<11.3; 2206 >11.3; 255
Chen, 2016	Pancreatology	NA	Mar 2015–Mar 2016	S	57/57	B, A, I, HL	<11.33; 30 >11.33; 27
Goyal, 2016	North Am J Med Sci	R	Jan 2009–Jun 2015	S	177/177	HTG, A	<11.33; 147 >11.33; 30
Párniczky, 2016*	PLoS One	P	Jan 2013–Jan 2015	M	113/600	B, I, A, A + D, HL, pE, O	<1.7; 59 1.7–5.64; 28 5.65–11.33; 4 ≥11.33; 22

First author, year	Source	Study design	Inclusionperiod	Centre	Patient number: used for analysis/total (n/n)	AP aetiology	Groups based on seTG (mM); patients (n)
Tai, 2016	Gastroenterol Res Pract	R	Feb 2010–Jan 2014	S	294/294	B, HTG	<11.33; 168 >11.33; 126
Sue, 2017	Pancreas	R	2006–2013	M	2519/2519	B, A, O	<1.7; 1729 1.7–2.26; 251 2.27–5.66; 308 5.67–11.32; 82 ≥11.33; 149
Wan, 2017	Lipids Health Dis	R	Jan 2005–Dec 2013	S	1539/1539	B, A, HTG, I, O	<1.7; 1078 1.7–2.23; 107 2.23–11.2; 242 >11.2; 112
Wu, 2017	Pancreatology	R	Jul 2009–Jul 2014	S	262/262	B, A, O	<1.7; 104 1.7–5.67; 72 5.67–11.33; 47 >11.33; 39
Zhu, 2017	Pancreas	NA	Jan 2005–Dec 2012	S	3203/3260	B, I, HL, A, O, (Mixed)	<11.33; 2736 >11.33; 467

Table 3. Characteristics of the studies included in the meta-analysis. A, alcoholic; B, biliary; I, idiopathic; M, multicentre; NA, not available; O, others; P, prospective; pE, post-endoscopic retrograde cholangiopancreatography; R, retrospective; S, single centre; seTG, serum triglyceride concentration. *The authors had access to the raw data in Párniczky et al. (2016) because of the overlap between the authors. Therefore, it was possible to create new groups based on seTG which were not presented in the original publication.

VI.2.3. Clinical outcomes

VI.2.3.1. Comparing the effects of hypertriglyceridemia vs. normal serum triglyceride on the severity of acute pancreatitis

Different groups were created based on the extent of HTG, and the outcomes for AP were compared with those in the normal (<1.7 mM) seTG group. Figure 16A shows how HTG affects the course of AP. HTG significantly increased the number of severe AP cases (severity), pancreatic necrosis, persistent OF and renal failure compared to the non-HTG group (Figure 16A). However, HTG did not significantly increase the odds for mortality and pulmonary failure compared to the <1.7 mM group. Analysing the effect of seTG in the range from 1.7 to 11.3 mM showed results similar to the previous comparison. The severity of AP and the incidence of POF significantly increased in the 1.7–11.3 mM range compared to the <1.7 mM seTG group, while it had no significant effect on the mortality of the patients (Figure 16B).

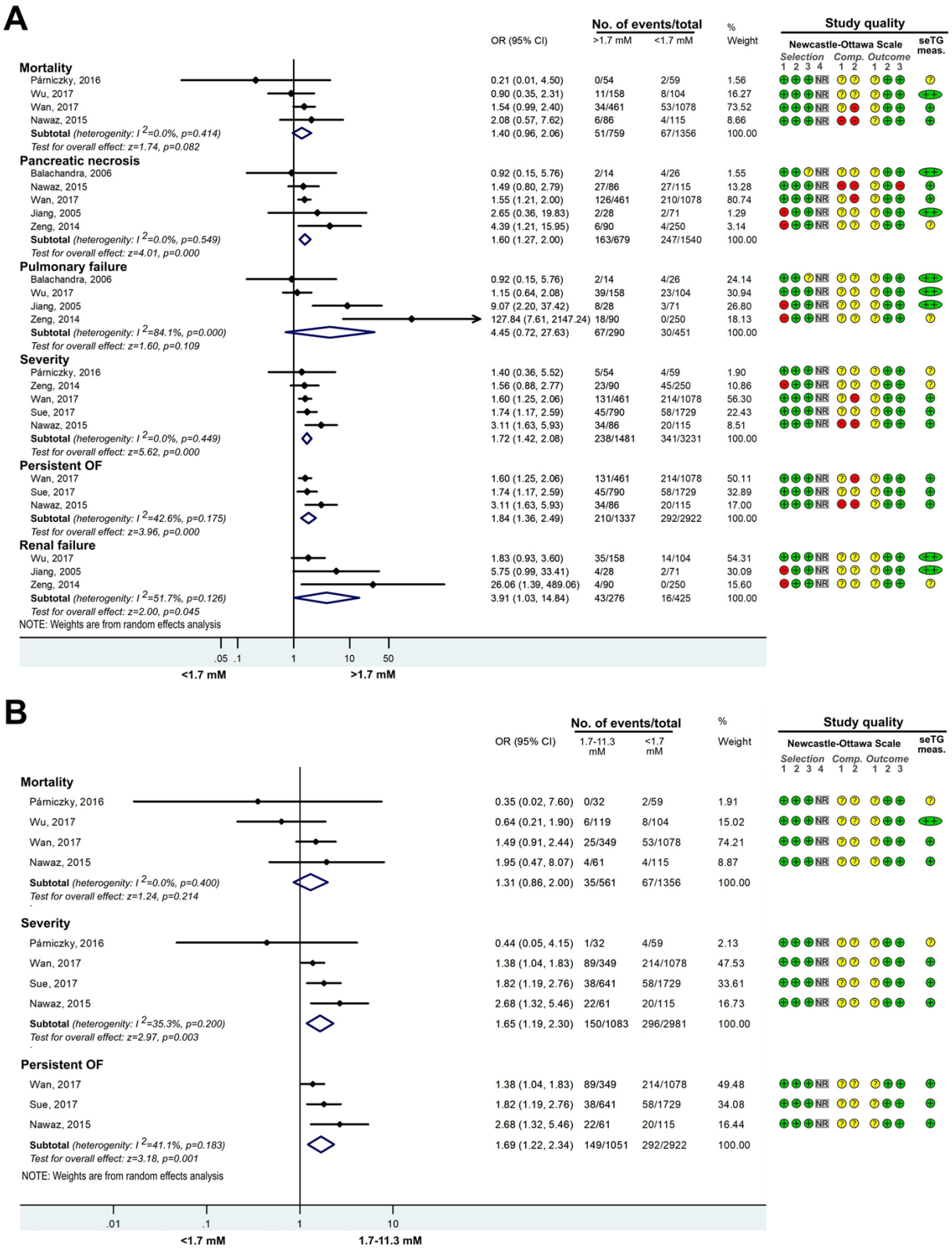

Figure 16. The effects of seTG >1.7 and 1.7–11.3 mM vs. <1.7 mM on AP severity, mortality, pancreatic necrosis, pulmonary and renal failure, and persistent OF. A, Forest plot shows the influence of seTG over 1.7 mM compared with normal seTG (<1.7 mM). B, The outcomes for the 1.7–11.3 mM seTG group were compared with those in patients with normal seTG (<1.7 mM). Filled rhombuses represent the risk ratio derived from the studies analysed. Horizontal bars represent 95% CI. Empty rhombuses show the overall effect (OR is the middle of the rhombus and CIs are the edges). CI, confidence interval; Comp., comparability; NR, not relevant; meas., measurement; OF, organ failure; OR, odds ratio.

HTG was further divided into ranges of 1.7–5.6, >5.6 and >11.3 mM seTG. Figure 17A shows that the severity of AP was not significantly different in patients with 1.7–5.6 mM seTG compared to the <1.7 mM group. However, seTGs >5.6 mM significantly increased the risk for severe AP in patients with OR of 2.01 [CI: 1.29–3.14; p=0.002] compared to seTG <1.7 mM (Figure 17B).

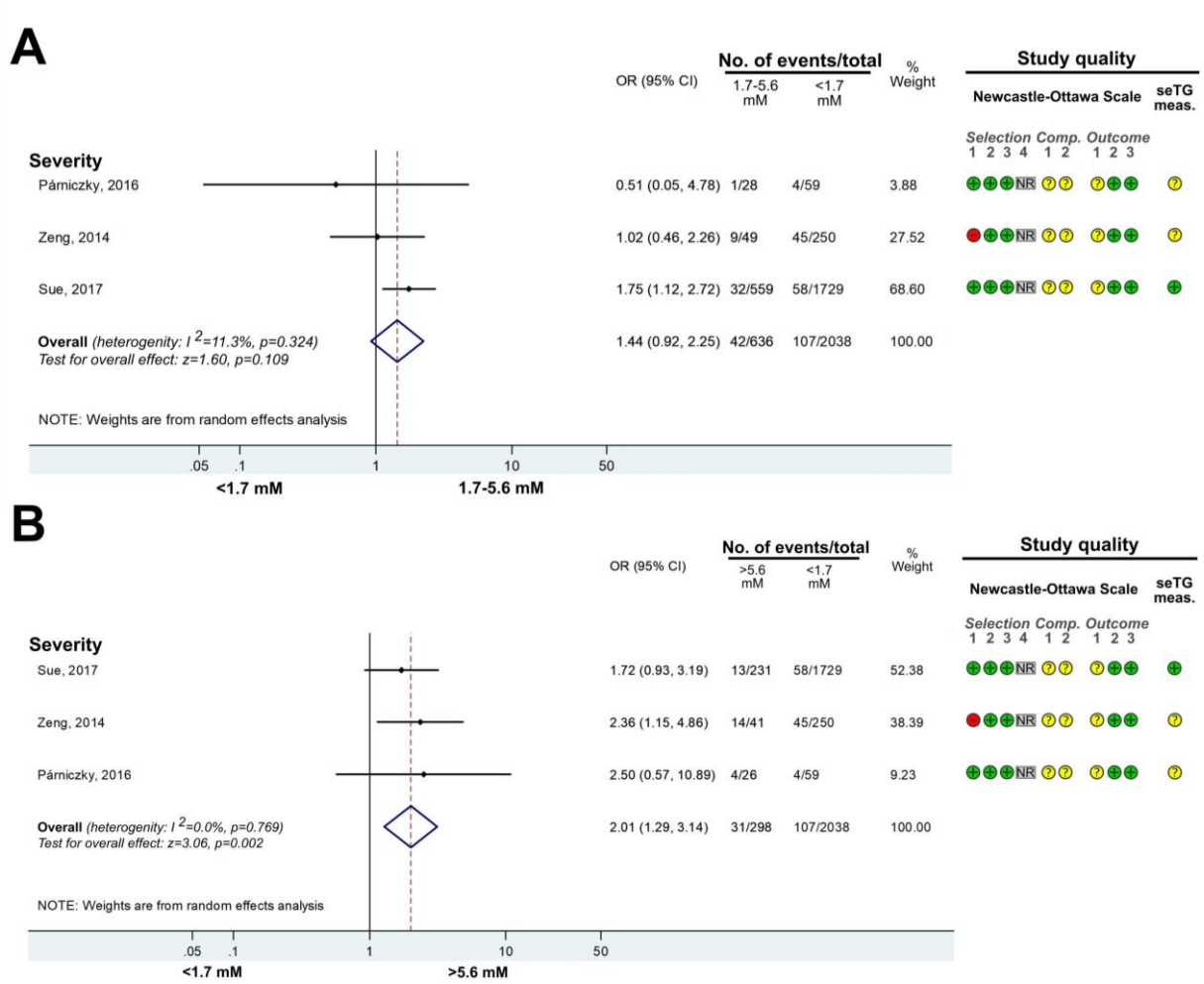

Figure 17. The effects of seTG at 1.7–5.6 mM and >5.6 mM vs. <1.7 mM on AP severity. A, Forest plot shows the influence of 1.7–5.6 mM seTG compared with normal seTG (<1.7 mM). B, The outcome of the >5.56 mM seTG groupwas compared with the outcomes of patients with normal seTG (<1.7 mM).

The presence of severe and very severe HTG (>11.3 mM) markedly increased the severity of AP (OR=3.08 [CI: 1.77–5.34; p=0.000]), POF (OR=2.39 [CI: 1.45–3.95; p=0.001]) and ICU admission (OR=3.90 [CI: 2.53–6.00; p=0.000]), but there was no significant elevation in mortality compared to the normal seTG group (Figure 18).

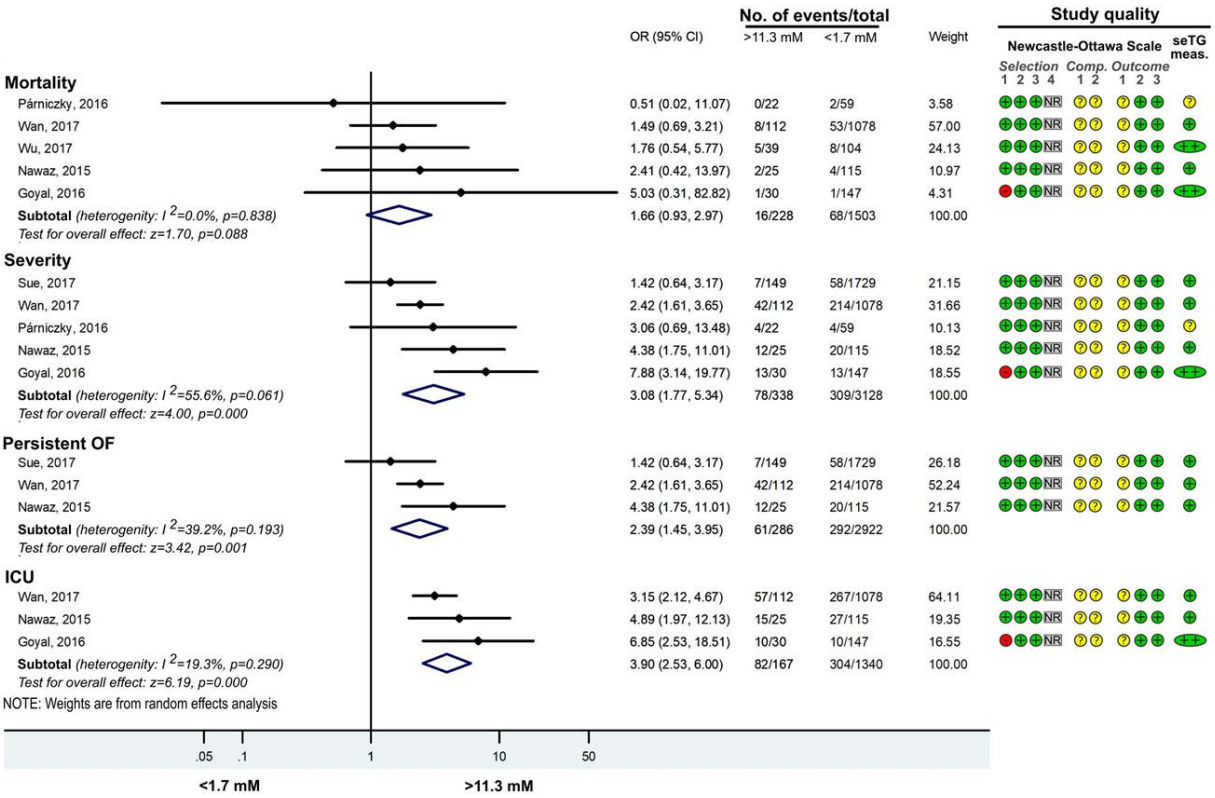

Figure 18. Forest plot showing the effect of seTG >11.33 mM vs. <1.7 mM on AP severity, mortality, persistent OF and the need for intensive care unit (ICU).

VI.2.3.2. The effect of different ranges of hypertriglyceridemia on acute pancreatitis

If seTG is elevated, the extent of the increase could also have an impact on the course of AP. Comparing the effect of seTG below and above 5.6 mM showed that seTG higher than 5.6 mM significantly increased the risk for severe AP, mortality, and pulmonary and renal failure (Figure 19A). However, the severity of AP was not significantly different in HTG patients with seTG of 1.7–5.6 mM vs. >5.6 mM (Figure 19B).

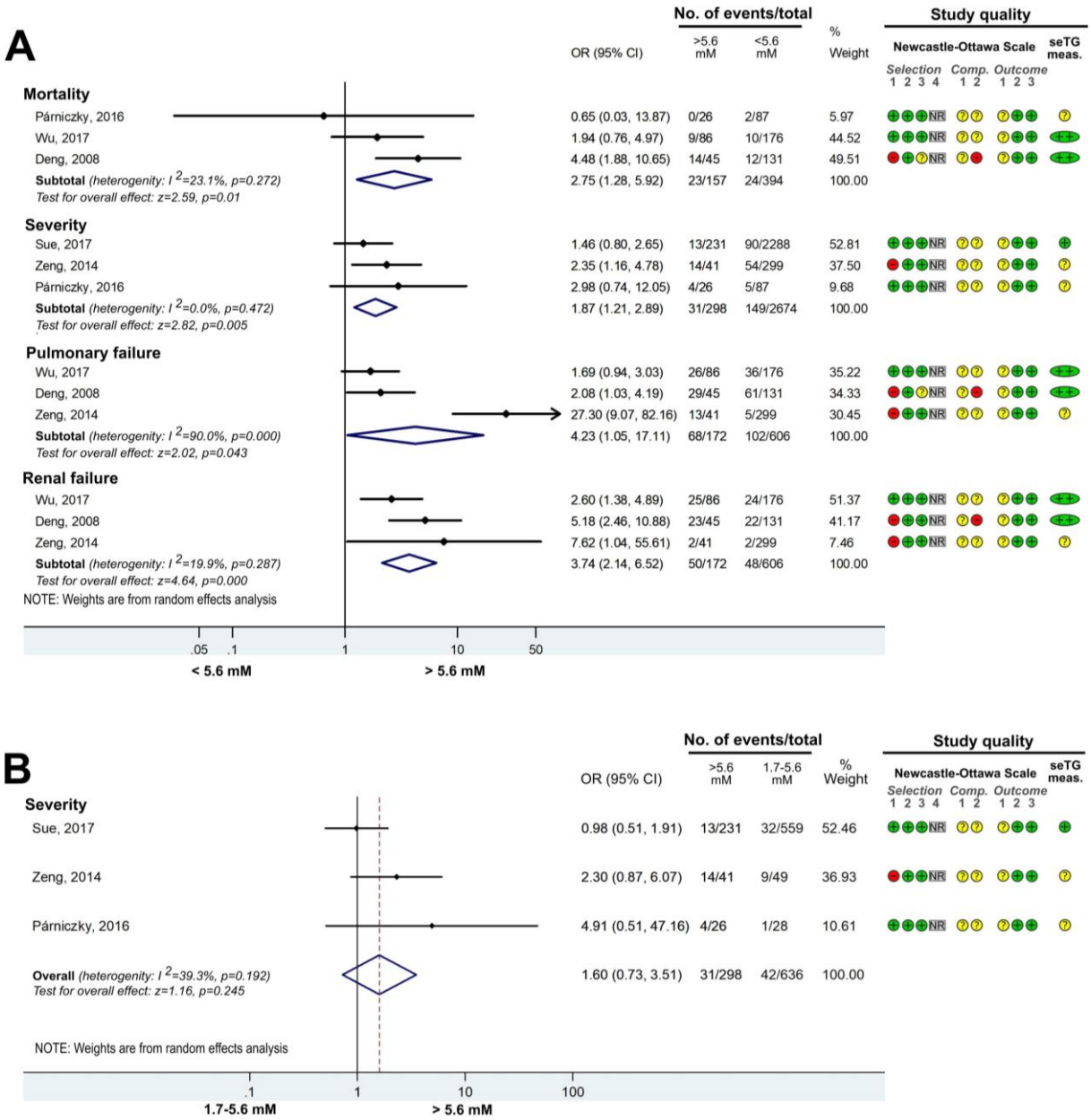

Figure 19. Forest plot showing the effect of seTG >5.6 mM vs. <5.6 or 1.7–5.6 mM on AP severity, mortality, and pulmonary and renal failure. A, Forest plot shows the influence of seTG over 5.6 mM compared with that of seTG <5.6 mM. B, The AP severity in the >5.6 mM seTG group was compared with that in patients with seTG in the 1.7–5.6 mM range.

Severe and very severe HTG (>11.3 mM seTG) significantly increased the OR of AP severity, mortality, pancreatic necrosis and ICU admission compared to group with seTG <11.3 mM, but it did not influence the occurrence of MOF (Figure 20).

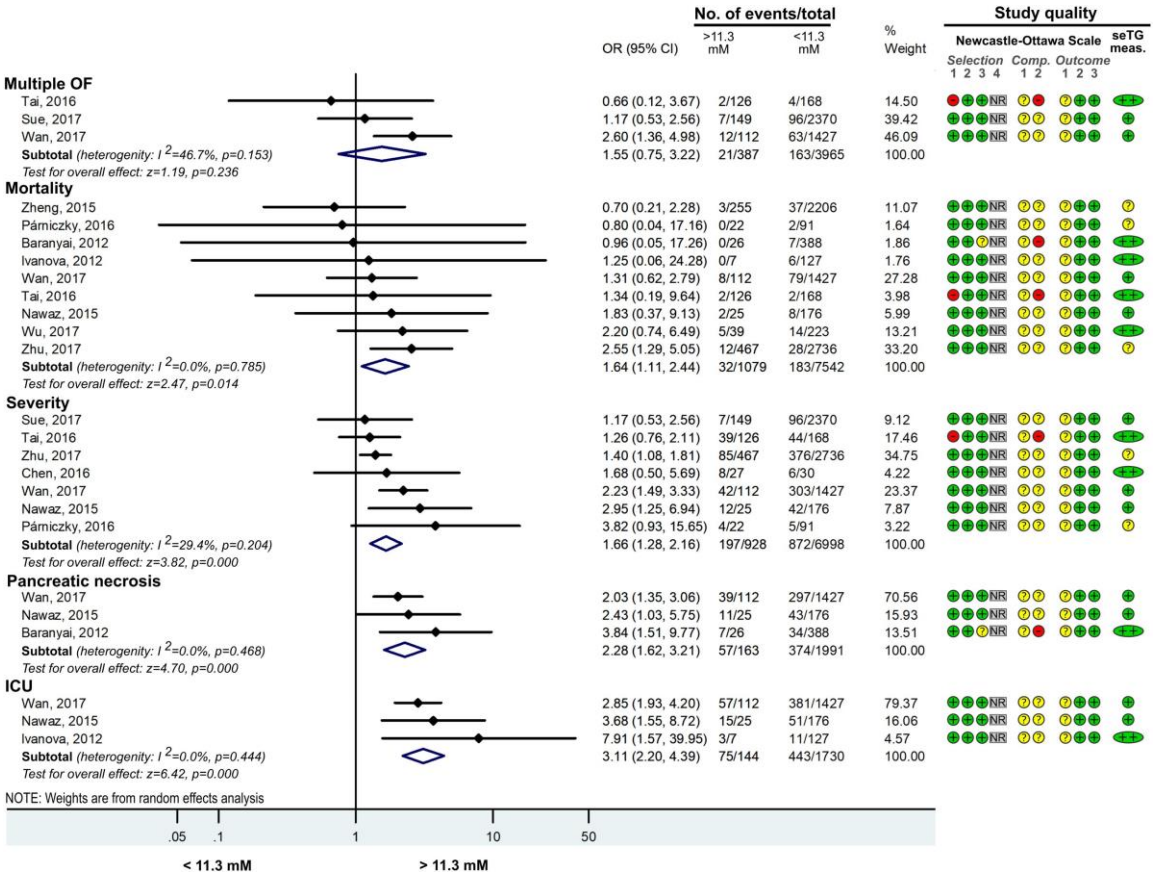

Figure 20. Forest plot showing the effect of seTG >11.3 mM vs. <11.3 mM on AP severity, mortality, pancreatic necrosis, the need for ICU admission and multiple OF.

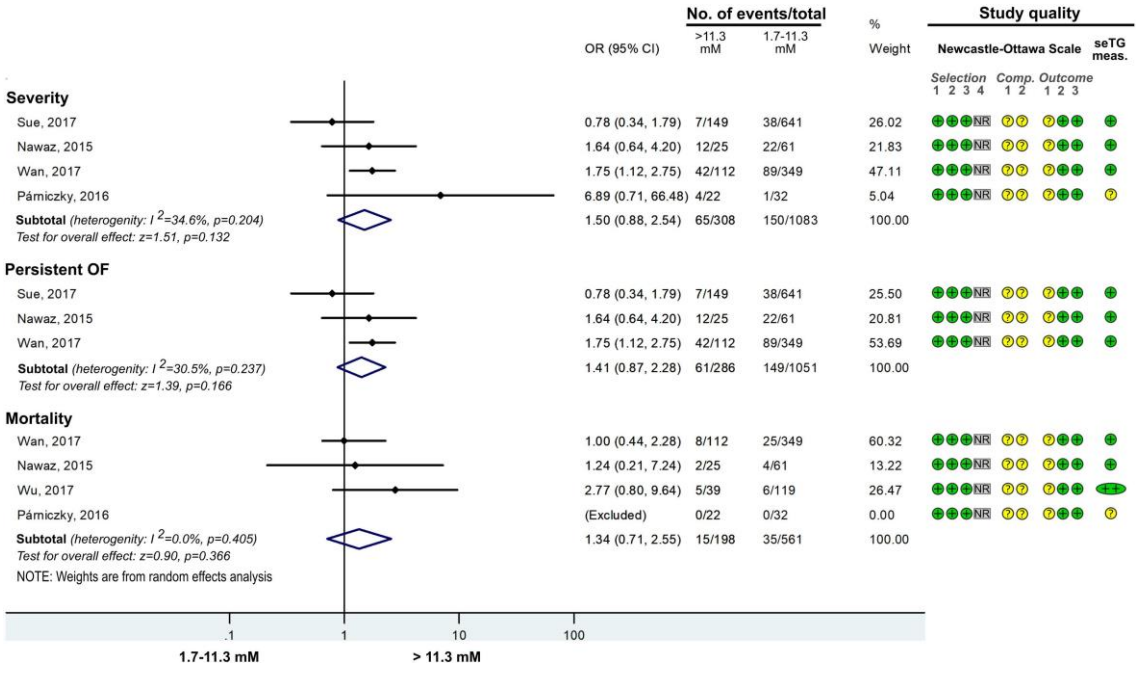

Figure 21. Forest plot showing the effect of seTG >11.3 mM vs. 1.7–11.3 mM on AP severity, mortality and persistent OF.

Interestingly, when the effect of severe and very severe HTG was compared with mild and moderate HTG (seTG 1.7–11.3 mM), no significant difference was revealed between the two groups with regard to AP severity, mortality and POF (Figure 21). All the groups compared on the basis of

seTG are summarized in Table 4. Data extracted from the articles were depicted on funnel plots to test the impact of small study effect. These plots are not listed here, but they can be found in the original article.

Comparison intervention	Interven-tion	Outcome OR [*CI; p*]							
		AP Severity	Mortality	Pancreatic necrosis	Persistent OF	Multiple OF	Pulmonary failure	Renal failure	ICU admission
<1.7	>1.7	**1.72** [*1.42–2.08; 0.000*]	**1.40** [*0.96–2.06; 0.082*]	**1.60** [*1.27–2.00; 0.000*]	**1.84** [*1.36–2.49; 0.000*]		**4.45** [*0.72–27.63; 0.109*]	**3.91** [*1.03–14.84; 0.045*]	
	1.7–11.3	**1.65** [*1.19–2.30; 0.003*]	**1.31** [*0.86–2.00; 0.214*]		**1.69** [*1.22–2.34; 0.001*]				
	1.7–5.6	**1.44** [*0.92–2.25; 0.109*]							
	>5.6	**2.01** [*1.29–3.14; 0.002*]							
	>11.3	**3.08** [*1.77–5.34; 0.000*]	**1.66** [*0.93–2.97; 0.088*]		**2.39** [*1.45–3.95; 0.001*]				**3.90** [*2.53–6.00; 0.000*]
<5.6	>5.6	**1.87** [*1.21–2.89; 0.005*]	**2.75** [*1.28–5.92; 0.01*]				**4.23** [*1.05–17.11; 0.043*]	**3.74** [*2.14–6.52; 0.000*]	
1.7–5.6	>5.6	**1.60** [*0.73–3.51; 0.245*]							
<11.3	>11.3	**1.66** [*1.28–2.16; 0.000*]	**1.64** [*1.11–2.44; 0.014*]	**2.28** [*1.62–3.21; 0.000*]		**1.55** [*0.75–3.22; 0.236*]			**3.11** [*2.20–4.39; 0.000*]
1.7–11.3	>11.3	**1.5** [*0.88–2.54; 0.132*]	**1.34** [*0.71–2.55; 0.366*]		**1.41** [*0.87–2.28; 0.166*]				

Table 4. Summary of the groups compared based on seTG. Black cells indicate significant differences between the groups ($p<0.05$), grey cells show no significant difference ($p>0.05$), and white cells stand for no comparison for that outcome. Under outcomes, the numbers in bold indicate the OR values and square brackets contain CI and p values for the respective comparisons.

VII. DISCUSSION

VII.1. Animal experiments

VII.1.1. CFTR and its restored function, as a central player in ameliorating AP severity

The pathomechanism of AP is complex and the underlying processes are not completely understood. However, the important role of ductal impairment and CFTR function in the pathomechanism of the disease is already known (Barreto et al., 2021; Hegyi & Rakonczay, 2015). Recently several drugs appeared commercially (e.g. VX-661 or VX-770) or are in clinical phases to restore impaired CFTR ion channel activity or the protein expression in diseases caused by mutations (e.g. cystic fibrosis). In our study, we found mislocalization of ductal CFTR during AP in mice, so the combination of VX-661 and VX-770 was applied to improve the function of pancreatic ducts during AP.

Pallagi et al. (2011) have shown that activation of trypsin in AP causes ductal CFTR inhibition through proteinase-activated receptor 2 and elevation of intracellular Ca^{2+} concentration. Our study demonstrates that not just functional inhibition, but also mislocalization of CFTR may cause the decrease of ductal function. Our results demonstrate that AP induces the loss of CFTR staining along the ductal lumen, and CFTR staining was observed in the perinuclear region. Presumably, the inflammation and cellular stress direct CFTR proteins into proteosomes for degradation (Ahner et al., 2013). Interestingly, the mRNA expression of *Cftr* was unchanged in the beginning of AP (6-12 h) and was significantly increased from 24 h. The protein expression of CFTR followed the mRNA changes and increased after 24 h. Similar results were found in an earlier study by Maléth et al. (2015). Human pancreata derived from patients with alcoholic AP and chronic pancreatitis were compared to normal pancreas. Alcoholic AP decreased *Cftr* mRNA and protein expressions in human samples, while chronic pancreatitis caused marked increase in mRNA expression and in cytoplasmic localization of CFTR proteins. In a cell culture and guinea pig AP model, Maléth et al. (2015) also demonstrated that ethanol and its metabolites decrease *Cftr* mRNA and protein expressions. Furthermore, ethanol and palmitoleic acid induced AP also caused CFTR mislocalization in guinea pig. Consequently, the results of the present study and our earlier investigation (Maléth et al., 2015) showed that two different animal models of AP cause CFTR mislocalization, suggesting that this adverse effect is independent of the disease aetiology.

The *ex vivo* HCO_3^- secretion of isolated mouse interlobular ducts (with a luminal diameter of 20-130 μm) was increased at 6 h after AP induction, whereas at later time points it was similar to that of the non-AP group. However, CFTR expression was not observed in these

ducts, only smaller (2-3 μm luminal diameter) intercalated ducts were stained for CFTR, as it was also shown by Burghardt et al. (2003) and Marino et al. (1991) in human samples. However, Fernandez-Salazar et al. (2004), Pallagi et al. (2014) and this work demonstrated functional CFTR activity in interlobular ducts by fluid secretion measurement. Therefore, it is likely that the CFTR expression in mouse pancreatic interlobular ducts is lower compared with intercalated ducts. Based on our results, the measured increase in ductal HCO_3^- secretion at 6 h after AP initiation mainly relates to activation of transporters other than CFTR, e.g. SLC26A3, SLC26A6, ANO1, NBC, NHE or H^+-ATPase. Previous publications have demonstrated that etiological factors like ethanol or its metabolites and bile acids concentration dependently stimulate or inhibit the function of ducts (Judák et al., 2014; Maléth et al., 2011 and 2015; Venglovecz et al., 2008). These investigations used ducts derived from guinea pig, which have prominent CFTR expression in interlobular ducts and could be activated by CFTR agonists (e.g. cAMP; Ishiguro et al., 2009).

Previous publications (Judák et al., 2014; Maléth et al., 2011 and 2015; Venglovecz et al., 2008) and the present work suggest that etiological factors of AP or the disease itself initiates CFTR mislocalization or degradation, and inhibition of fluid secretion. These factors possibly contribute to increased pancreatic inflammation. Therefore, we hypothesized that pharmacological correction of ductal function should reduce pancreatic damage and acinar necrosis/apoptosis in AP. For this reason, the combination of CFTR corrector, VX-661 and potentiator, VX-770 was used. Pre-treatment of mice with VX-661+VX-770 significantly decreased the pancreatic tissue damage during AP; however, other inflammatory parameters were similar to the AP group. Interestingly, the expression and localization of CFTR protein was not changed by the VX-661+VX-770 treatment. Since the expression of CFTR was unchanged after VX-661+VX-770 treatments, we suppose that the residual and functional CFTR proteins in pancreatic ducts were activated, and this could lead to the observed decrease in acinar damage. As localization of CFTR protein was unchanged, we think that the beneficial effect in AP was mainly related to the use of CFTR potentiator (VX-770).

We could demonstrate that CFTR correction and potentiation by VX molecules increases fluid secretion in pancreatic ducts isolated from AP mice. We hypothesize that ductal secretion defends the pancreas by washing out toxic agents like activated digestive enzymes. If this defence mechanism is insufficient, the harmful agents cannot be eliminated from the pancreas, and this can result in tissue damage. Previously, Niderau et al. (1985) and Renner et al. (1983) showed that fluid secretion stimulated by secretin has protective effects in AP. Furthermore, galanin inhibits basal bicarbonate secretion which exacerbates the inflammation of pancreas

(Brodish et al., 1994; Hegyi et al., 2011). Overall, it seems that fluid secretion is an important protecting mechanism which can be enhanced in AP by VX-661+VX-770 treatment.

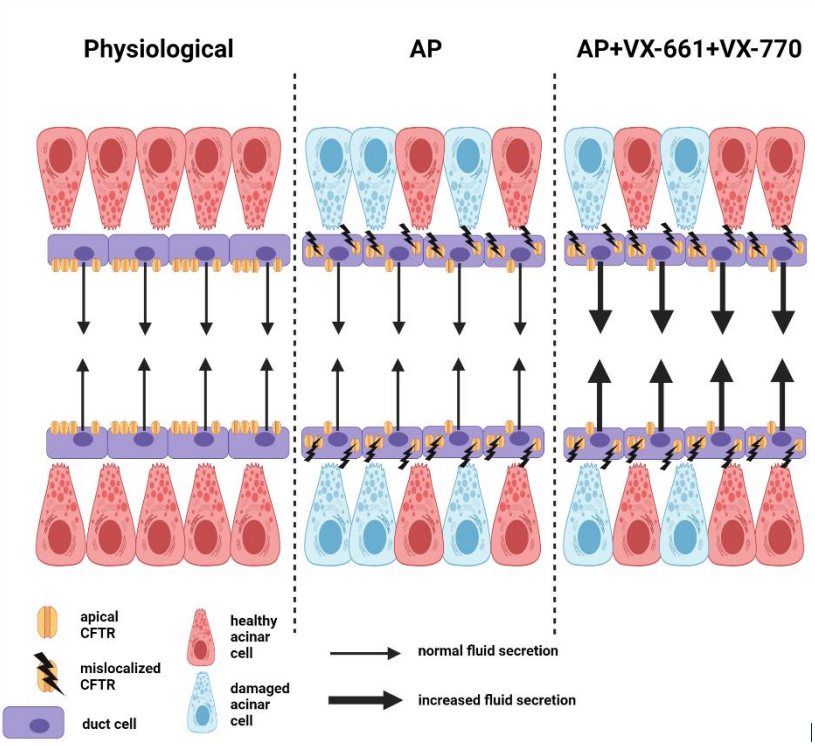

Figure 22. The role of CFTR in the pancreas under physiological and pathological conditions. Under physiological conditions CFTR is mainly localized to the apical membrane of pancreatic duct cells. Cer-AP induces CFTR protein mislocalization in duct cells and damage of acinar cells in mice. VX-661 and VX-770 pre-treatment enhances ductal fluid secretion, but the localization of CFTR protein remained mainly cytoplasmic. Interestingly, the administration of VX-661 and VX-770 reduces the extent of acinar injury.

Zeng M et al. (2017) in autoimmune pancreatitis also successfully applied a CFTR corrector (C18) and VX-770, which reduced the extent of inflammation. They observed that increasing CFTR expression (C18) and activity (VX-770) enhanced ductal fluid secretion and clearance of the inflammation to allow repair of cell damage. However, they found that the correction by C18 caused the majority of the effects, and the effect of VX-770 was negligible in that model. In our study, we applied only one AP model, which is a limitation of this work. The secretagogue model utilized in the present investigation damages mainly the pancreatic acinar cells. It would be interesting to test the efficacy of CFTR correction or potentiation in a model where the damage of ducts is likely to initiate the disease. The intraductal NaTc-AP model could be used for this reason, which is a well-accepted model of biliary pancreatitis.

VII.1.2. The effect of fentanyl on the severity of acute pancreatitis

Opioids are used to relieve the pain in AP, but the literature is divided on whether administration of these drugs are beneficial or detrimental. Our published study is a multi-objective comparative work, in which we investigated the effects of different analgesics (FE, MO or BQ), in three animal models (NaTc, L-ornithine or Cer) of AP. This thesis focuses on

the results about FE in the NaTc-AP model.

FE pre-treatment in the NaTc model greatly exacerbated the condition of the animals, therefore after humanely terminating the experiments this type of treatment was discontinued. FE pre-treatment in L-ornithine-induced AP increased the severity of the disease but the animals survived, whereas in Cer-AP model, the drug had no effect (other parts of our work are published in Bálint et al., 2022). The post-treatment of FE in necrotizing models of AP (NaTc and L-ornithine) ameliorated the disease. In NaTc-AP the higher dose (0.2 mg/kg) of FE, whereas in the L-ornithine model even the lower dose (0.1 mg/kg) of FE significantly reduced the severity of AP. This leads to the conclusion that in mild form of the disease, FE treatment does not affect the outcome, but in severe necrotizing pancreatitis, the timing of medication is important.

Experimental and clinical studies also reported the beneficial effects of FE. Wang & Chen (2017) tested the effects of intravenous FE administration on NaTc-induced AP. They injected FE 23–23.5 h after AP induction and sacrificed the animals 24 h after the induction of the disease. Surprisingly, FE reduced pancreatic inflammation and AP-induced myocardial damage within that really short time (30–60 min). Furthermore, FE restored the AP-reduced microcirculatory flow after epidural administration, which resulted in decreased tissue damage and the occurrence of systemic complications (Demirag et al., 2006; Sadowski et al., 2015). However, the potential problems related to opioids include its inhibitory effects on intestinal motility with resultant anorexia, constipation, nausea and vomiting, also habituation and abuse (Wang D, 2017). A recent experimental trial demonstrated that administration of MO, exacerbated the severity of AP and increased the risk for bacterial translocation (Barlass et al., 2018; Mahapatra et al., 2019). MO also delayed macrophage migration and caused a persistence of inflammation (Barlass et al., 2018). Opioids exert their effects on mu, kappa, or delta opioid receptors, which are expressed on neuronal or immune cells. Their affinity or specificity to their receptors are different. Trescot et al. (2008) showed that FE is 80 times more potent than MO and is a highly selective full MOR agonist ligand. Therefore, FE can also suppress the immune system (Franchi et al., 2019). MO and FE can alsocause a sphincter of Oddi spasm, which could further aggravate AP severity (Afghani et al., 2017).

VII.2. Meta-analysis of the effect of serum triglyceride concentration on the outcome of acute pancreatitis

HTG (>1.7 mM) on admission worsens the course of AP compared to the normal seTG group. However, increase in seTG up to 5.6 mM did not significantly influence the severity of AP compared to normal seTG. Selecting subgroups within HTG (>5.6; >11.3 mM) resulted in a significant elevation of ORs (2.01 and 3.08, respectively) for severity when all the groups were compared with the effect of normal seTG. Our findings are in line with earlier animal studies, in which hyperlipidaemia increased the severity of AP (Czakó et al., 2007; Hofbauer et al., 1996; Noel et al., 2016; Wang YJ et al., 2006; Wang Y et al., 2009).

Interestingly, the mortality of patients did not show statistically significant differences between >1.7; 1.7-11.3; 11.3 mM groups compared to the normal, which is likely to be the result of the small number of patients with this outcome. The odds for complications (SIRS, POF, involving pulmonary, renal, and circulatory failure) were significantly increased in groups with HTG. Although mortality is related to disease complications, the results from the statistical analysis for mortality were not in line with the outcomes for AP (e.g. severity, POF, necrosis). Therefore, further investigation would be beneficial for the relation of HTG and AP with respect to mortality.

Although there is no unanimous definition for HTG-AP, it is widely accepted that AP with seTG >11.3 mM is HTG-related (Gelrud & Whitcomb, 2016). However, some researchers consider HTG-AP to be defined by a seTG threshold >5.6 mM (Carr et al., 2016). Therefore, this encouraged us to investigate the relationships between the extent of HTG and the outcome of AP. SeTG >5.6 mM significantly worsened the outcomes for AP when compared with the seTG <5.6 mM group, while there was no difference when seTG >5.6 mM was compared with seTG in the1.7–5.6 mM range. Similar results were seen at a cut-off seTG of 11.3 mM. SeTG >11.3 mM caused more severe AP than seTG <11.3 mM, but when the effect of seTG >11.3 mM was compared with that of seTG in the 1.7–11.3 mM range, no significant difference was seen between the two groups. These comparisons also support our previous assumption that compared to normal seTG, HTG is associated with an increased risk for severe AP and complications. Further studies would be important to clarify the relationship between the extent of HTG and the severity of AP.

Most of the earlier attempts to investigate the effect of HTG on the severity of AP via meta-analysis were unsuccessful due to the small number of available observational studies (Carr et al., 2016; Murad et al., 2012), except for Wang Q et al. (2017). It analysed 14 articles,

five of which overlapped with studies included in our paper (Wang Q et al., 2017). A careful look at this publication revealed that inconsistent data and grouping abound. Two groups were defined for the comparisons, the TG-related AP (TGAP) group and the non-TG-related AP (NTGAP) group. The patients categorized as TGAP had seTG > 11.3 mM or >5.6mM with a previous history of HTG. Otherwise, patients were categorized into the NTGAP group. However, we are unsure how this categorization relates to their statistical analysis because in some of the articles involved the seTGs were classified as <2.26 or <1.7 mM and used for the NTGAP group, and the TGAP group contained patients with seTG > 2.26 or >1.7 mM. Furthermore, some errors could also be identified for the patient numbers used for the analysis. Thus, Wang Q et al. (2017) compared two groups of patients, one with seTG > 1.7 mM and one with seTG < 5.65 mM, where the two groups overlap between 1.7 and 5.65 mM seTG. The authors proved that the AP group with higher seTG had an increased risk for systemic complications and an elevated mortality rate compared to patients with lower seTG. Our results partly confirm the findings from Wang Q et al. (2017).

In 2016 and 2017, eight well-written articles (Chen et al., 2016; Goyal et al., 2016; Párniczky et al., 2016; Sue et al., 2017; Tai et al., 2016; Wan et al., 2017; Wu et al., 2017; Zhu et al., 2017) were published in which the effect of HTG on the severity of AP was investigated, and this allowed us to prepare a meta-analysis by combining those cohorts with those in earlier papers. However, our study has several limitations: (1) Although the literature is more extensive nowadays, most of our analyses contained a small number of articles (generally 3–5 studies). (2) The different populations (China or the USA) and the various baseline data (e.g. body mass index, age and sex) could represent a bias. (3) Aetiologies (biliary or alcoholic) for AP differ. (4) Only English-language articles were included in this study, which can affect the results. (5) There were no statistical significances for all the investigations even if the ORs were high (e.g. OR = 4.45 with p value of 0.109). A further increase in the number of articles and patients could clarify any discrepancies related to ORs or significance. (6) Significant heterogeneities were detected for some analyses. (7) Notably, seTG changes dynamically, which is related to food intake and fasting. Current treatment protocols for most AP patients include fasting at the beginning of hospitalization, except for suspected severe AP cases where early enteral feeding (within 48 hours) is recommended (Greenberg et al., 2016; Vege, 2018). Fasting results in a rapid (within 48 hours) drop of seTG (Carr et al., 2016) and measuring seTG 48 or 72 hours after the admission might underrepresent levels at the onset of AP. Dominguez-Muñoz et al. (1991) demonstrated a dramatic decrease in seTG during fasting:

seTG falls from approx. 30 mM to 5 mM within three days. Other authors also confirmed this phenomenon (Carr et al., 2016; Lloret Linares et al., 2008; Tsuang et al., 2009). To take this bias into account, we scored the articles based on the timing of the seTG measurement. Having high scores for NOS and seTG measurement timing represents good quality for the selection of articles for this meta-analysis.

To improve the design of further retrospective or prospective cohorts related to HTG and to AP, we would suggest some recommendations. It is advisable to keep the time interval for the seTG measurement consistent. Preferably, it should be performed within 48 hours after the onset of the first symptoms and repeated regularly. Publishing the medical history of patients with respect to seTG-lowering therapies would also be advantageous, e.g. describing the regular use of statins or fibrates, which could decrease the incidence of AP (Ivanova et al., 2012). If patients have HTG-AP on admission, then lipid-lowering therapy (such as plasmapheresis) is recommended according to guidelines. The use of these therapies would also improve further studies. Due to large differences between the kits used for seTG determination, standardizing the method for seTG measurement is also recommended. Based on basic discoveries, cohort analyses, clinical studies and meta-analyses, early intervention (e.g. heparin and/or insulin therapies and plasmapheresis) to normalise HTG may be beneficial for patients, and this should be investigated in randomised controlled trials.

VII.3. Conclusions

We demonstrated that AP markedly affects the expression and function of the CFTR channel in pancreatic ducts. Correction and stimulation of the CFTR channel was shown to be beneficial, as the VX drug combination significantly improved pancreatic cell damage and ductal bicarbonate secretion rates in pre-treatment of AP mice compared to control groups. We also showed that FE treatment could effectively decrease the severity of necrotizing AP, but the timing of drug administration is important. Our meta-analysis of clinical studies confirmed that HTG worsens the severity of AP and increases the odds of complications. Overall, it seems that numerous factors have roles in the development and aggravation of AP. Therefore, an appropriate combination of treatments can be the answer to curing the disease.

VIII. SUMMARIES

VIII.1. Summary of thesis

Introduction: Besides pancreatic acinar cells, ductal epithelial cells also have a prominent role in the pathogenesis of AP. The most important function of ducts is the secretion of HCO_3^-, which is highly dependent on the appropriate function of ion channels and transporters (e.g. CFTR, SLC26). The development or exacerbation of AP may be influenced by the disfunction of these transporters, or by many other factors, such as HTG, excessive alcohol consumption, and biliary obstruction. Until now, there is no specific treatment for the disease, only supportive management is available, such as pain reduction by analgesics. In the literature there is no consensus about which types of painkillers can be recommended. Opioids are widely used and these are the most potent drugs against pain. However, these can exert side effects along with other types of analgesics.

Aims: Our overall goal was to study the effects of different factors on AP by using experimental animal models and processing clinical data. One of our specific aims was to investigate how AP affects the pancreatic ductal CFTR Cl^- channel; furthermore, we tested how CFTR correction and potentiation can influence the disease progression. Among the opioids, the effect of FE on the severity of AP was examined. Moreover, by using meta-analysis and systematic review, we wanted to demonstrate how seTG concentrations affect AP severity and the associated complications.

Methods: Necrotizing AP was induced with ip. administration of Cer in mice or i.d. administration of NaTc in rats. In Cer-AP, first we monitored the animals for three days at different time points, and evaluated disease-specific laboratory and histological parameters, examined CFTR protein expression and morphological changes by immunohistochemistry, and mRNA expression of different ductal transporters, as well as functional changes of isolated pancreatic ductal fragments. In the second experimental setup, we stimulated CFTR expression and function with VX-661+VX-770 pre-treatment and their effects on AP severity were investigated. In NaTc-AP model the effects of FE pre- or post-treatments were tested on AP severity. To determine the effect of seTG on the outcome of AP in patients, data were collected to carry out a meta-analysis in which TG levels were compared (normal vs. elevated or different elevated levels). A literature search was performed in PubMed, Embase and ClinicalTrial.gov and all relevant literature was processed. Primary outcome was severity, secondary outcomes were mortality, pancreatic necrosis, pulmonary failure, renal failure, persistent organ failure, multiple organ failure and intensive care unit admission.

Results: Cer-induced AP reached its peak severity at 12 and 24 hours with excessive tissue damage. Interlobular ducts showed increased HCO_3^- secretory activity at 6 h after AP initiation. At the later time points structural regeneration of the tissue was observed and the ductal CFTR expression and localization started to recover, which could be confirmed by immunohistochemistry. Pre-treatment with VX-661 and VX-770 reduced the rate of cellular damage in AP and improved ductal CFTR function. Pre-treatment of rats with FE greatly exacerbated the outcome of necrotizing AP, whereas FE post-treatment decreased necrosis, leukocyte infiltration and serum amylase activity. In humans, HTG seems to worsen the severity of AP in a concentration-dependent manner. Even seTG concentrations between 1.7 and 11.3 mM can aggravate AP. Above 5.6 mM seTG, the risk of death and various organ failure is increased. Above or equal to 11.3 mmol/l, the disease is more often associated with pancreatic necrosis and the need for hospitalisation in intensive care unit.

Conclusions: We demonstrated that AP markedly affects the expression and function of the CFTR channel in pancreatic ducts. Correction and stimulation of the CFTR channel was shown to be beneficial, as the VX drug combination significantly improved pancreatic cell damage and ductal bicarbonate secretion rates in pre-treatment of AP mice compared to control groups. We also showed that FE treatment could effectively decrease the severity of necrotizing AP, but the timing of drug administration is important. Our meta-analysis of clinical studies confirmed that HTG worsens the severity of AP and increases the odds of complications. Overall, it seems that numerous factors have roles in the development and aggravation of AP. Therefore, an appropriate combination of treatments can be the answer to curing the disease.

VIII.2. Summary of new findings

- Experimental AP causes mislocalization of CFTR protein, while it increases *Cftr* mRNA expression.
- The CFTR corrector and potentiator, VX-661 and VX-770, significantly reduce the extent of pancreatic tissue damage possibly via increased fluid secretion, but the CFTR protein expression was unchanged.
- NaTc-AP combined with FE post-treatment reduced, while pre-treatment exacerbated the severity of the disease.
- Elevated seTG concentrations significantly increase the severity of AP in human patients, with a higher likelihood of death, organ damage and hospitalisation. HTG as an aetiological factor of AP deserves particular attention.

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"The researcher knows what frustration is, knows how many months of working in the wrong direction, and knows the failures. But failures are also useful, because if you analyse them correctly, they can lead to success." — Sir Alexander Fleming

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XII. ANNEX