INVESTIGATION OF THE EFFECTS OF AETIOLOGICAL FACTORS AND THERAPEUTIC AGENTS ON THE SEVERITY OF ACUTE PANCREATITIS

Ph.D. Thesis

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PUBLICATIONS

Articles closely related to the subject of the thesis

I. **Bálint ER**, Fűr G, Kui B, Balla Z, Kormányos ES, Tóth B, Horváth G, Pallagi P, Maléth J, Venglovecz V, Hegyi P, Kiss L, Rakonczay Z Jr. Fentanyl but not morphine or buprenorphine treatment improves the severity of necrotizing acute pancreatitis in rats. Int J Mol Sci. 2022; 23(3):1192. PMID: 35163111 **[IF_{2020}: 5.924]**.

II. Tóth E, Maléth J, Závogyán N, Fanczal J, Grassalkovich A, Erdős R, Pallagi P, Horváth G, Tretter L, **Bálint ER**, Rakonczay Z Jr, Venglovecz V, Hegyi P. Novel mitochondrial transition pore inhibitor N-methyl-4-isoleucine cyclosporin is a new therapeutic option in acute pancreatitis. J Physiol. 2019; 597(24):5879-5898. PMID: 31631343 **[IF_{2019}: 4.547]**.

III. **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 Jr.. Assessment of the course of acute pancreatitis in the light of aetiology: a systematic review and meta-analysis. Sci Rep. 2020; 10(1):17936. PMID: 33087766 **[IF_{2020}: 4.379]**.

Articles not closely related to the subject of the thesis

I. Szentesi A, Tóth E, **Bálint E**, Fanczal J, Madácsy T, Laczkó D, Ignáth I, Balázs A, Pallagi P, Maléth J, Rakonczay Z Jr, Kui B, Illés D, Márta K, Blaskó Á, Demcsák A, Párniczky A, Pár G, Gódi S, Mosztbacher D, Szücs Á, Halász A, Izbéki F, Farkas N, Hegyi P; Hungarian Pancreatic Study Group. Analysis of research activity in gastroenterology: pancreatitis is in real danger. PLoS One. 2016; 24(11)10:e0165244. PMID: 27776171 **[IF_{2016}: 3.057].**

II. Demcsák A, Lantos T, **Bálint ER**, Hartmann P, Vincze Á, Bajor J, Czopf L, Alizadeh H, Gyöngyi Z, Márta K, Mikó A, Szakács Z, Pécsi D, Hegyi P, Szabó IL. PPIs are not responsible for elevating cardiovascular risk in patients on clopidogrel-a systematic review and meta-analysis. Front Physiol. 2018; 19(9):1550. PMID: 30510515 **[IF_{2018}: 3.289]**.

III. Pécsi D, Farkas N, Hegyi P, Varjú P, Szakács Z, Fábián A, Varga G, Rakonczay Z Jr, **Bálint ER**, Erőss B, Czimmer J, Szepes Z, Vincze Á. Transpancreatic sphincterotomy is effective and safe in expert hands on the short term. Dig Dis Sci. 2019; 64(9):2429-2444. PMID: 31055720 **[IF_{2019}: 2.751]**.

IV. Tóth B, Hegyi P, Lantos T, Szakács Z, Kerémi B, Varga G, Tenk J, Pétervári E, Balaskó M, Rumbus Z, Rakonczay Z, **Bálint ER**, Kiss T, Csupor D. The efficacy of saffron in the treatment

of mild to moderate depression: a meta-analysis. Planta Med. 2019; 85(1):24-31. PMID: 30036891 **[IF$_{2019}$: 2.758]**.

V. 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 Cs, 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. 2021; 12:702764. PMID: 34745090 **[IF$_{2020}$: 7.561]**.

VI. 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 Jr Z. Mislocalization of CFTR expression in acute pancreatitis and the beneficial effect of VX-661 + VX-770 treatment on disease severity. J Physiol. 2021; 599(22):4955-4971. PMID: 34587656 **[IF$_{2020}$: 5.182]**.

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I. INTRODUCTION

I.1. Physiology of the pancreas

The pancreas functions both as exocrine and endocrine gland. The endocrine part regulates blood glucose concentration by secreting hormones (insulin, glucagon, somatostatin, etc.). The exocrine part of the pancreas secretes 1.5-2 litres of isotonic fluid containing digestive enzymes and high concentrations of HCO_3^- (up to 140 mM). The two most important cell types of the exocrine pancreas are acinar and ductal cells. Acinar cells produce digestive proenzymes and Cl^--rich isotonic fluid. Digestive proenzymes are packed in zymogen granules within the cells. After secretion, enzymes reach the gut lumen, where enterokinase catalyses the conversion of trypsinogen to trypsin. Then trypsin further activates other trypsinogen molecules as well as other proenzymes. Ductal cells secrete HCO_3^--rich isotonic fluid. The physiological function of this fluid is to prevent premature activation of trypsinogen inside the ductal lumen, to wash out the digestive enzymes of the ductal tree into the duodenum and to provide optimal pH for the function of the digestive enzymes by neutralizing gastric acid.

I.2. Acute pancreatitis

I.2.1. Incidence, aetiology

Acute pancreatitis (AP) is one of the most common reasons for hospitalization in case of gastrointestinal diseases, which has an overall mortality of about 2%. The incidence of the disease is more than 30 per 100 000 population in Europe and this number has shown increasing tendency over time.

Gallstones represent the main aetiological background of AP globally (approx. 40%), which are diagnosed by imaging techniques and liver function tests. Gallstone-related or biliary AP (BAP) occurs twice as often as alcohol-induced AP (AAP). AAP is caused by regular, excessive alcohol consumption usually with a clinical history of >5 years and >50-100g/day. Hypertriglyceridaemia (HTG) with serum triglyceride concentrations >11.3 mM is the third most common (approx. 9%) known aetiological factor of the disease. Less frequent causes of AP include endoscopic retrograde cholangiopancreatography (ERCP), hypercalcaemia, pancreas divisum, tumours, genetic polymorphisms and drugs. To date, no standardized diagnostic criteria exist for post-ERCP AP (PAP). The guidelines recommended by Cotton *et al.* are most commonly applied, which suggest PAP to be diagnosed if pancreatitis develops within 24 h after the procedure.

I.2.2. Pathomechanism

The factors mentioned above induce pathological Ca^{2+} signaling in the pancreas, which triggers premature trypsinogen activation, nuclear factor kappa B (NF-κB) activation, decreased ductal HCO_3^- and fluid secretion, inhibition of digestive enzyme secretion, decreased blood flow in the gastrointestinal tract,

inhibition of cystic fibrosis transmembrane conductance regulator, decrease of intracellular ATP level, opening of mitochondrial permeability pore (mPTP), loss of mitochondrial transmembrane potential, mitochondrial damage and endoplasmic reticulum stress. Mitochondrial damage and decreased ATP levels in acinar and ductal cells lead to cell death.

To date, cyclosporin A (CYA) is the only licensed substance applied experimentally to inhibit mPTP. A non-immunosuppressive CYA derivative, NIM811 has been shown to have a favourable pharmacokinetic profile and similar oral bioavailability as CYA. NIM811 has been reported to be beneficial in both experimental and clinical settings. No adverse reactions occurred during the studies where NIM811 was applied.

I.2.3. Diagnosis and treatment

The diagnosis of AP requires the presence of at least two of the following three features: abdominal pain, at least a threefold increase in serum amylase/lipase activity, characteristic findings on contrast-enhanced computed tomography or ultrasonography. To date, there is no specific therapy for the disease. The initial treatment is supportive including fluid replacement, nutrition and analgesia. As pain is the most prominent symptom of AP, its relief is priority in clinical settings. Unfortunately, recent guidelines for AP treatment do not have clear recommendations for the types of analgesics to be used. Most commonly, the WHO pain management guideline is utilized and treatment ranges from nonsteroidal anti-inflammatory drugs (NSAID) to high potent opioids. The latter are applied in cases of severe AP and include fentanyl (FE), buprenorphine (BQ), pethidine, pentazocine, morphine (MO) etc. Although opioids are the most effective pain killers which makes them valuable in clinical settings, there is a scientific debate on their use due to their side effects like constipation or immunosuppression. The use of MO is often not preferred in humans since it induces spasm of the sphincter of Oddi, which might worsen the outcome of AP. In addition, Barlass *et al.* have shown the drawbacks of MO use in AP in a mouse model.

I.2.4. Classification of severity

Based on the Revised Atlanta Classification (RAC), AP severity can be categorized into three groups: mild, moderately severe and severe. Although the majority of cases are mild with a self-limiting course, the mortality rate of severe AP can reach 30% which underlies the desperate need of finding proper treatment. Organ failure (OF) is the most important determinant of this classification system. Patients with mild AP have no organ dysfunction and usually recover within a week. Moderately severe AP resolves slower and might require interventions because of the presence of transient organ failure (<48 h). Severe AP results in persistent organ failure (POF) which lasts >48 h. Multiple organ failure (MOF) is defined as failure of two or more organ systems, which can be transient or persistent. The three extrapancreatic organs most commonly affected by AP are the lungs, the heart and the kidneys. Approximately 25% of AP patients develop severe complications and have to be admitted to an intensive care unit (ICU). About 25-30% of

patients experience recurrent AP, which refers to a clinical condition defined by repeated episodes of AP. Recurrent AP has a high risk of progression to chronic pancreatitis or pancreatic cancer.

Although there are several risk factors, it is difficult to predict which patient will develop mild, moderately severe or severe AP. To date, numerous clinical studies have investigated the effect of aetiology on AP progression. However, to the best of our knowledge, there have been no efforts to summarize clinical data on how various aetiological backgrounds affect the severity and course of AP.

II. AIMS

The main aims of this work were to assess the effects of analgesia and mitochondrial protection on the course of AP. Furthermore, we wanted to reveal the predisposing effect of aetiology on AP severity. Our detailed aims were the following:

- to investigate the effects of opioid (FE and MO) administration (pre-or post-treatments) on the course of AP in rats in different disease models.
- to test the effects of the novel CYA derivative NIM811 on the severity of AP during *in vivo* experiments in mice.
- to reveal the impact of the aetiological factors for AP on disease severity by performing thorough literature search and meta-analysis on available clinical data.

III. MATERIALS AND METHODS

III.1. Animals

Wistar rats were used for the experiments related to opioid treatment, while mPTP was targeted in C57Bl/6 J mice. All experiments were performed in compliance with the European Union Directive 2010/63/EU and the Hungarian Government Decree 40/2013 (II.14.). Experiments were approved by both local (University of Szeged) and national ethics committees (X/3354/2017 and XII/4988/2015) for investigations involving animals.

III.2. Materials

All chemicals were purchased from Sigma-Aldrich (Budapest, Hungary) unless indicated otherwise.

III.3. Experimental setup

III.3.1. The effect of analgesia on the severity of acute pancreatitis

Necrotizing AP was induced by a single intraperitoneal (i.p.) injection of 3 g/kg L-ornithine-HCl (LO, 30%, pH=7.4). Oedematous AP was induced by hourly i.p. injections of 20 µg/kg caerulein (CER, 50 µg/ml) four times. Control groups were given physiological saline (PS: 0.9% NaCl) solution instead of LO/CER.

FE was administered at doses of 0.1 and 0.2 mg/kg based on literature data. Different timing arrangements were applied for FE in various AP models. In addition, FE was used as pre- or post-treatment. 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 CER- or 11 h in LO-induced AP, respectively. In the post-treatment setup, animals received the first FE injection 1 h after AP induction in case of the LO-model or 0.5 h after AP induction in case of the CER-model.

In the post-treatment setup, 5 mg/kg MO was administered i.p. 8 times every 2 h in case of the LO-model. The dose and timing of MO was chosen based on literature data. During pre-treatment, 10 mg/kg MO was injected i.p. 9 times every 2 h. When AP was induced by CER, 4x5 mg/kg dose of MO was used i.v. every 2 h and analgesia was started simultaneously with AP induction.

III.3.2. The mitochondrial transition pore as potential therapeutic target in acute pancreatitis

Mild AP was induced by the combination of 1.75 g/kg ethanol and 750 mg/kg palmitic acid i.p. in mice. NIM811 (MedChem Express Europe, Sollentuna, Sweden) was gavaged orally 1 h prior to AP induction in the pre-treatment setup and 12 h after AP induction in the post-treatment groups at doses of 5 and 10 mg/kg.

III.3.3. Termination of experiment, tissue collection

At the end of experiments, deep anaesthesia was induced by pentobarbital injection (85 mg/kg i.p. for the rats and 200 mg/kg for the mice; Bimeda MTC, Cambridge, Canada). Rats were sacrificed 24 or 12 h after AP induction with LO or CER, respectively. Mice were sacrificed 24 h after AP induction. Blood was collected through cardiac puncture, then the pancreas was rapidly removed. Pancreata were cleaned on ice from fat and lymph nodes. Two parts of the pancreatic tissue were immediately frozen in liquid nitrogen and stored at –80 °C until use. The third part of the pancreas was fixed in 8% neutral formaldehyde solution for histological analysis. Blood samples were centrifuged at 2500 RCF for 15 min at 4°C, the sera were collected and stored at -20 °C until use.

III.3.4. Laboratory measurements

Serum amylase activity was measured on a Fluorostar Optima plate reader (BMG Labtech, Ortenberg, Germany) with a colorimetric kinetic method using a commercial kit purchased from Diagnosticum ZRt. (Budapest, Hungary). To evaluate pancreatic water content, the wet/dry weight ratio of the pancreata was calculated. Pancreatic myeloperoxidase (MPO) activity was measured according to Kuebler *et al.* MPO activities were normalized to total protein content as measured by the Lowry method. To determine the extent of inflammatory response in the pancreata, we measured IL-1β levels by a commercial ELISA kit from R&D Systems (Minneapolis, MN, USA) as described by the manufacturer.

III.3.5. Histological examination

Formalin-fixed pancreatic tissues were sectioned to 3 μm. Prepared sections were stained with hematoxylin and eosin, then analysed and scored by two independent experts blinded to the experimental protocol. Oedema was scored between 0-3 points, leukocytic infiltration between 0-4 points, vacuolisation between 0-3 points and the percentage of acinar cell damage was also evaluated.

III.3.6. Statistical analysis for experimental data

Data are presented as means ± SEM. Experiments were evaluated by one- or two-way ANOVA followed by Holm–Sidak post hoc test (SPSS, IBM, Armonk, NY, USA). $P<0.05$ was accepted as statistically significant.

III.4. Meta-analysis

III.4.1. Protocols applied

Our systematic review and meta-analysis followed the recommendations of Stroup *et al.* and was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The analysis was based on the Problem, Intervention, Comparison intervention and Outcome (PICO) model as follows: AP patients with alcoholic, biliary, hypertriglyceridaemic and post-ERCP aetiologies were compared in order to examine the effect of aetiologies on disease outcomes. Primary outcome was severity, secondary outcomes were POF, MOF, ICU admission, recurrence rate, mortality and pancreatic necrosis. The protocol for the meta-analysis was registered in the PROSPERO database on 05/15/2018 (https://www.crd.york.ac.uk/PROSPERO/, ID: CRD42018093574).

III.4.2. Search strategy

Literature search was conducted in the electronic databases Embase and Pubmed from publication

date 01/01/2012 to 05/31/2018. The reason for the start date is that the RAC was introduced in 2012, which provides the most accepted and widespread criteria for determining AP severity. Due to the limitations of the length of the thesis, only the results of AAP, BAP and HTG-AP patients are analysed here. For further details please see our article published in Scientific Reports. The search was restricted to studies written in English or in Hungarian.

III.4.3. Eligibility criteria

All randomised trials, retrospective and prospective cohort studies were included that involved adult patients with AP and relevant data are categorized according to the aetiology of the disease. Four major disease backgrounds were included: alcohol abuse, HTG, biliary disease and post-ERCP. Articles that studied only one aetiological group or compared one aetiological group with another group called others or non-… (e.g. alcohol vs. non-alcohol) were excluded. Non-human studies or articles with data from patients younger than 18 years of age were not included. In case of cohort overlap between studies, only the most recent study was included unless a prior study had higher quality.

When assessing AP severity, only studies were included where severity was defined according to the RAC, because in this case it was crucial to present a consistent and clear definition for the analysis. Articles were also excluded if only one or two of the three severity groups were analysed. Both local complications and OFs could lead to serious conditions and death which are characteristic features of moderately severe and severe AP. Therefore, these two groups were combined in our study, and are referred to as “non-mild” disease forms and compared to the mild group. In cases of outcomes other than severity, using only the RAC was not in the criteria.

III.4.4. Study selection and data extraction

Titles and abstracts of publications were screened independently by two review authors to identify studies that potentially meet inclusion criteria. The full texts of these potentially eligible studies were also independently assessed for eligibility by the same two review authors. Disagreement between reviewers was resolved by discussion with other two colleagues. Two review authors independently extracted study characteristics (author, title, journal, study location, inclusion period, number of centres involved, type of study, number of participants) and outcome data (severity, POF, MOF, ICU admission, recurrence rate, mortality, pancreatic necrosis), which were recorded on a standardized Microsoft Excel spreadsheet. Discrepancies were resolved by discussion.

III.4.5. Data analyses

Statistical analysis was performed with Stata 11 SE (StataCorp LLC, College Station, TX, USA). Odds ratios (ORs) calculated from patient numbers were used to compare outcomes in different aetiologic groups. Summary OR estimation, p value and 95% confidence interval (CI) were calculated. $P<0.1$ was

considered as significant difference from summary OR=1. BAP is defined as primary reference group, the other aetiologies are ranked in the following order: AAP, HTG-AP.

Statistical heterogeneity was analysed using the I^2 statistic and the chi-square test to acquire probability values; $p<0.1$ was defined to indicate significant heterogeneity. The small-study effect (in case of comparisons with at least 10 articles) was visually investigated on funnel plots and was also confirmed by Egger's test. Sensitivity analysis was performed to examine the robustness of our results.

IV. RESULTS

IV.1. Animal experiments

IV.1.1. The effect of fentanyl pre-treatment on acute pancreatitis severity in rats

The pancreata of the control group displayed normal morphology and FE alone did not induce any structural changes in the rat pancreas. When the higher dose (3x0.2 mg/kg) of FE was applied, the extent of tissue necrosis significantly increased, whereas the level of leukocyte infiltration was higher in the 3x0.1 mg/kg FE & AP group compared to the AP group not receiving FE. FE pre-treatment did not cause any change in pancreatic water content in the AP groups. 3x0.1 mg/kg FE significantly increased serum amylase activity, while pancreatic MPO activity was greatly elevated due to the dose of 3x0.2 mg/kg FE in AP. The concentration of pancreatic IL-1β significantly decreased due to 3x0.1 mg/kg FE treatment in the AP group.

I.p. injections of CER induced mild AP and increased the extent of pancreatic vacuolisation, leukocyte infiltration and oedema compared to the control group. FE pre-treatment did not cause any change during AP progression in histological parameters or water content. CER-induced AP resulted in elevated serum amylase activity and pancreatic IL-1β content, whereas it did not significantly affect MPO activity. FE pre-treatment did not alter serum amylase activity or pancreatic IL-1β level in the AP groups.

IV.1.2. The effect of fentanyl post-treatment on acute pancreatitis in rats

In contrast to FE pre-treatment, both doses of FE post-treatment decreased the extent of histopathological changes (leukocyte infiltration and pancreatic tissue damage) caused by LO-induced AP in rats. On the other hand, FE administration did not alter pancreatic water content in the AP groups. Serum amylase and pancreatic MPO activities were decreased by both FE doses in AP. Pancreatic IL-1β levels only decreased significantly in case of the LO + 3 x 0.2 mg/kg FE group.

I.p. injections of CER increased the extent of pancreatic vacuolisation, leukocyte infiltration, and tissue water content causing mild oedematous AP. FE treatment did not affect either histological parameters (pancreatic damage, leukocyte infiltration) or pancreatic water content. The elevated serum amylase and pancreatic MPO activities during AP were unaffected by FE post-treatment. Interestingly, the

smaller dose of FE (0.1 mg/kg) further increased the elevated pancreatic IL-1β during AP, while the higher dose of FE had no effect.

IV.1.3. Morphine administration does not affect the severity of acute pancreatitis

MO alone did not induce any structural changes in pancreatic tissues of rats at the tested doses, and no inflammatory cell infiltration could be observed in histological sections. During LO-induced AP it did not significantly alter the value of histopathological parameters (tissue necrosis, leukocyte infiltration), pancreatic water content, serum amylase or pancreatic MPO activities in the AP groups either in pre- or post-treatment groups. In the CER-induced oedematous AP MO was administered simultaneously with CER. In this case, MO significantly reduced acinar vacuolisation but it had no effect on leukocyte infiltration, pancreatic water content, serum amylase or pancreatic MPO activities.

IV.1.4. NIM811 has a protective effect against ethanol+fatty acid-induced acute pancreatitis in mice

The EtOH + FA treatment significantly increased serum amylase levels and the scores of pancreatic necrosis, oedema and leukocyte infiltration compared to the absolute control group in mice. The higher dose of NIM811 (10 mg/kg) pre-treatment significantly reduced most symptoms of AP examined by us (elevated serum amylase levels, necrosis and leukocyte infiltration), whereas oedema was not influenced by it. In case of NIM811 post-treatment, the lower dose of NIM811 (5 mg/kg) improved pancreatic leukocyte infiltration and oedema, while serum amylase activity or the extent of pancreatic necrosis did not differ significantly.

IV.2. Meta-analysis

IV.2.1. Study selection

The search strategy identified 11,288 records. After removing duplicates 7,733 articles were retrieved. Out of these, 456 records seemed to be relevant to the study question based on screening by title or abstract. After assessing the articles in full text, 329 records had to be excluded with different reasons. Finally, 127 publications fulfilled the eligibility criteria.

IV.2.2. Characteristics of studies included

The majority of the included cohort studies (108 out of 127) collected data from the 2010's. Our meta-analysis contains 102 single- and 23 multicentre studies. In two cases, there were no relevant data regarding the number of centres involved. Sample sizes ranged from 11 to 1,165,777. Only the data of the four types of AP (AAP, BAP, HTG-AP, PAP) were analysed. Due to the limitations of the thesis, the detailed characteristics of the included studies or the quality assessment are not included here. Please find

the details in our publication in Scientific Reports.

IV.2.3. Clinical outcomes

IV.2.3.1. Severity

HTG proved to induce non-mild AP in a significantly higher number of cases than the other aetiological factors. Alcoholic aetiology significantly increased AP severity compared to biliary-related events. We found heterogeneity in comparison of AAP vs. BAP. No signs of small-study effect could be detected in comparisons of AAP vs. BAP, AAP vs. HTG-AP, BAP vs. HTG-AP.

IV.2.3.2. Organ failures and intensive care unit admission

There was no significant difference in the occurrence of POF or MOF between AAP and BAP. The frequency of ICU admission was also similar in AAP and BAP patients. Heterogeneity was found in case of ICU admission.

IV.2.3.3. Recurrence rate

Recurrence rate was significantly higher in AAP vs. BAP patients and in HTG-AP vs. BAP patients. However, AP did not reoccur more frequently due to alcoholic aetiology than HTG or post-ERCP. Heterogeneity was found in all comparisons except for the comparison between HTG-AP and AAP. No signs of small-study effect could be detected when recurrence rate was compared in AAP and BAP groups.

IV.2.3.2. Mortality and pancreatic necrosis

Mortality rate proved to be significantly higher in HTG-AP than in AAP, but no statistical difference was found between any other patient groups. In the comparison of AAP and BAP, a large proportion of patients came from one study contributing 1,165,777 subjects (accounting for 12.76% weight). However, sensitivity analysis showed that the results remained similar when this study was excluded. Pancreatic necrosis was reported more often in AAP than BAP patients. No significant difference was detected in any other comparisons regarding necrosis. Heterogeneity was found in the comparison of mortality rate between BAP and HTG-AP, AAP and BAP, and in case of necrosis when AAP and BAP groups were compared.

V. DISCUSSION

V.1. Animal experiments

V.1.1. The effect of opioids on the severity of acute pancreatitis in rats

Opioids are commonly used for pain control in AP patients. It has been speculated that these analgesics (such as morphine) may affect AP progression. Therefore, we comprehensively investigated the effects of FE on the severity of experimental AP, and this research was further supplemented with the examination of the effects of MO. It is important to note that measurements were performed when experimental AP reached its maximal severity.

I.p. FE pre-treatment significantly increased the severity of necrotizing AP induced by LO, but it had no effect on oedematous AP evoked by CER. Interestingly, the clinically more relevant post-treatment with FE either decreased or had no effect on the various parameters of AP severity in different models. MO pre- or post-treatment did not affect the severity of the disease in case of LO-induced necrotising AP. Furthermore, simultaneous administration of MO and CER had no remarkable effect on disease progression either, except for vacuolisation, which was decreased by MO.

Opioids exert their effects primarily through mu, kappa, or delta opioid receptors which are expressed mainly by neuronal or immune cells. The effects can differ depending on their affinity or specificity to certain receptors. Publications showed that MO has immunosuppressant properties through full mu receptor agonism. MO treatment results in inhibition of cytokine production, NK cell activity, cellular responses to mitogens, antibody production, cell growth, and decreased phagocytic activity. FE, an 80 times potent MO analogue is a full mu receptor agonist, similarly to MO. Therefore, it can also suppress the immune system. Both MO and FE can cause Sphincter of Oddi spasm, which could further aggravate AP severity. Only FE pre-treatment resulted in increased AP severity. The early immunosuppression by FE may cause this adverse effect, while FE post-treatment was beneficial for AP outcome. However, later consequences were not investigated by this work. For all clinically applied opioids, including FE and MO, these effects should be considered and investigated in future studies. Moreover, timing of opioid administration can be critical, especially in case of FE.

In the clinical setting, there are no guidelines or recommendations suggesting the best opioid to use in AP. However, application of effective analgesics is necessary in the treatment of this disease. In light of the results discussed above, post-treatments (eg. FE, MO) do not increase disease severity, but some of the opioids (eg. MO) may affect the resolution of AP. Therefore, the latter may not be the best treatment option in this severe disease. Our results showed that FE post-treatments have promising effects besides pain relief; therefore, its use could also be beneficial on AP severity. Overall, this research contributes to the better understanding of the opioid effect in AP and can help in planning further clinical trials which will be necessary to choose the most appropriate opiate for treatment of this potentially lethal disease.

Although pre-treatment with analgesics in AP is clinically less relevant, in case of endoscopic

retrograde cholangiopancreatography (ERCP) rectal administration of non-steroid anti-inflammatory drugs (NSAIDs, e.g. indomethacin or diclofenac) is indicated. These agents reduce the development of PAP. In this case, opiates should be used with caution.

V.1.2. The mitochondrial transition pore as potential therapeutic target in acute pancreatitis

Mitochondrial damage is one of the key pathophysiological events in the early phase of AP. Over the past decade, both genetic and pharmacologic inhibition of mPTP has been shown to reduce BA- or EtOH+FA-induced acinar cell damage and alleviate AP severity. Numerous mPTP inhibitors have been tested, none of which proved beneficial. As an exception, NIM811 has been found to be advantageous in several diseases without adverse effects. *Per os* administration of 5 or 10 mg/kg NIM811 significantly alleviated the severity of experimental AP. However, it is interesting that the higher dose of NIM811 was effective in pre-treatment, while the lower dose proved beneficial in post-treatment. Further investigations are needed to find out the underlying mechanisms. These results suggest that mitochondrial function and restoration of cellular energy production are key features in AP. Our team was the first to confirm that NIM811 is a potential compound to be tested in clinical trials for AP. Phase 2 clinical trials should be set up to test the utility of this promising drug candidate in human medicine.

V.2. Meta-analysis

We performed the first detailed meta-analysis investigating the relationship between different aetiologies (alcohol abuse, biliary, HTG, post-ERCP) and the course of AP. Our study revealed that the prevalence of severe and moderately severe (non-mild) disease forms was highest in case of HTG-AP which was followed by AAP and BAP and PAP. Due to the large number of included articles and patients, our results have strong evidence in case of the severity outcome. These are also in accordance with our earlier observations and the data of Wang *et al*. One of the possible pathomechanisms is that lipotoxicity mediated by unsaturated fatty acids contributes to necrosis, OF (eg. cardiovascular diseases) and mortality. Experimental studies also demonstrated that HTG exacerbates the severity of AP. Fatty acid administration resulted in elevated intracellular Ca^{2+} concentration in pancreatic acinar cells and impaired mitochondrial function. HTG-AP is often accompanied by one or more secondary factors (alcoholism, medications, uncontrolled diabetes mellitus, physical inactivity), which can further aggravate the severity of the disease. Furthermore, elevated serum chylomicron concentration during HTG increases viscosity, causing reduced blood flow in microvessels and resulting in ischemic conditions. This could be an additional risk factor for a severe form of AP.

Determining the exact aetiology of AP may be challenging in some cases. For example, alcohol is not only known as an independent risk factor for AP, but it can also increase serum TG concentrations. In addition, mild-to-moderate elevation in TG concentrations can be observed in the early phase of AP, regardless of aetiology. Since serum TG concentrations can rapidly decrease during fasting state after the diagnosis of AP, the measurement of TG concentrations on (or shortly after) admission is crucial.

The number of events, which refer to positive outcomes in certain aetiologies were relatively high in case of severity and partly in mortality and recurrence rate outcomes. Smaller number of events could be included in the analysis of other outcomes. Low event rates can have detrimental influence on the reliability of the results. Based on the studies mentioned above, the results of all severity comparisons, mortality and recurrence rates in comparisons of AAP vs. BAP are strongly reliable. Most of the other calculations have lower reliability but there is no precedent to contradict the results of severity.

VI. SUMMARY OF NEW FINDINGS AND CONCLUSIONS

FE post-treatment proved to be beneficial in experimental AP. Our results suggest that type, dosing, administration route and timing of opioid treatment can influence the effects on AP outcome.

Our experiments proved that NIM811 had no adverse effects on the pancreas and is definitely beneficial in experimental AP.

We found association between aetiology and the development and course of AP. HTG proved to carry the highest risk for non-mild (moderately severe and severe) AP, which was followed by AAP; the least severe disease forms were observed in BAP and PAP. It is essential to determine the cause of the disease in time to apply the most appropriate therapy. Based on the results, greater emphasis should be placed on determining aetiology on admission, especially in case of HTG-AP.

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”It always seems impossible until it’s done.” — Nelson Mandela