P0084 EXPRESSION OF INSULIN AND GLUCAGON IN LIVER AND PANCREAS IN RAT COPPER-DEFICIENT MODEL OF INJURY

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Introduction: The copper-deficient model of injury is one for studying pancreas regeneration, where we can see destruction of pancreatic parenchyma, concomitant liver injury and regeneration of both organs during the recovery phase of the experiment.
Aims & Methods: The aim of our work was to study the expression of insulin and glucagon in pancreas and liver tissue in rat copper-deficient model of injury. 24 white Wistar male rats (80–100 g weight) were maintained on copper-deficient diet (MP Biomedicals, USA) containing a relatively non-toxic copper-chelating agent, triethylenetetramine tetrahydrochloride in final concentration of 0.6% w/w for 8 weeks, and then returned to normal rat chow for another 8 weeks (recovery phase). Groups of 3 animals each were killed after 2, 4, 6, and 8 weeks of copper-deficient diet and 2, 4, 6, and 8 weeks of recovery phase. Paraffin sections of liver and pancreas were stained immunohistochemically using antibodies to insulin and glucagon. The level of these hormones was measured in venous blood of rats.
Results: After 4 weeks of copper-deficient diet we observed glucagon-positive cells in peripheral part of the islets as well as single glucagon-positive cells and their clusters resembling newly formed islets. Expression of glucagon was maximal after 8 weeks of diet and continued to be at the same level 2 and 4 weeks after rats were returned to normal rat chow. Groups of glucagon-positive hepatocyte-like cells were found in the pericentral areas of liver, solitary glucagon-positive hepatocyte-like cells in liver parenchyma during all weeks of our experiment. The maximum of glucagon expression in liver was detected 2 weeks after return to normal rat chow. Glucagon-positive cells were observed only in peripheral part of the pancreatic islets after 6 and 8 weeks of recovery phase. Expression of glucagon in liver decreased alongside; glucagon-positive cells were located predominantly in pericentral areas. Insulin-positive cells were found in central part of the pancreatic islets during the experiment. Insulin-positive hepatocyte-like cells were detected in the pericentral areas and in liver parenchyma from 4^{th} to 6^{th} weeks of diet, the number of these cells was the highest after 6 weeks of copper-deficient diet. After 8 weeks of diet we observed insulin-positive small round cells – at least 1 positive cell was around every tenth of central vein (these cells were single after 4 and 6 weeks of diet). Appearance of the insulin-positive hepatocyte-like cells was accompanied with increase of insulin level in venous blood. The maximum of insulin expression in liver coincided with the highest concentration of insulin in venous blood after 6 weeks of diet. After rats were transferred to normal rat chow, we observed solitary insulin-positive small round cells in liver tissue until 2^{th} week. Later positive cells were not detected in liver.
Conclusion: We suppose that glucagon-positive pancreatic islet cells are probably the source of pancreas regeneration in rat copper-deficient model; the appearance of glucagon- and insulin-positive cells in liver confirms common origin of these organs and can be the response reaction for the pancreatic tissue injury.
Disclosure of Interest: All authors have declared no conflicts of interest.

P0085 A NEW ANIMAL MODEL IN PANCREATOLOGY- PANCREATIC DUCTAL FLUID AND BICARBONATE SECRETION IN WILD TYPE FERRETS

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Introduction: Cystic fibrosis (CF) is a lethal genetic disease affecting several organs, including the pancreas. Although animal models are available to study the CF-related tissue damage they have clear limitations. Recently a cystic fibrosis transmembrane regulator (CFTR) knock out ferret model was generated. This model would be the first available one to study pharmacological prevention of the disease development.
Aims & Methods: We aimed to characterize the fluid and bicarbonate secretion of wild type (WT) ferret pancreatic ducts. Expression of CFTR was detected by immunohistochemistry. Intra/interlobular pancreatic ducts were isolated from the WT ferret pancreas. Resting pH, buffer capacity and Cl^-/HCO_3 exchange activity were evaluated by microfluorometry. Fluid secretion was examined by video microscoopy.
Results: CFTR was expressed on the luminal membrane of ferret pancreatic ducts. The resting intracellular pH of pancreatic epithelial cells is lower (7.17 ± 0.08) in ferrets compared to mice (7.31) or to guinea pigs (7.36). Concerning the bicarbonate influx mechanisms, functionally active sodium/hydrogen exchanger and sodium/bicarbonate cotransporter were detected. Anion exchanger activity measured by NH_4Cl^- technique, Cl^- removal and inhibitory stop methods indicated that ferret pancreatic ducts secrete similar amount of bicarbonate as mice and guinea pigs. Video microscopy revealed a significant increase in fluid secretion to HCO_3^- and to 5 μM forskolin stimulation.
Conclusion: Ferret pancreatic ductal epithelial cells express the major epithelial ion transporters. Our results indicate that ferret could be a suitable model organism to study the CF-related pancreatic damage. Moreover this model opens up the possibilities to test pharmacological interventions in the disease development.
Disclosure of Interest: All authors have declared no conflicts of interest.

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P0086 CYCLIC ADENOSINE MONOPHOSPHATE PRODUCTION STIMULATED BY ORAI1 AND EXTENDED SYNAPTOTAGMIN 1

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Introduction: Cyclic adenosine monophosphate (cAMP) and Ca^{2+} signaling play central role in the regulation of the secretory functions of epithelial cells. The two signaling system have multiple synergistic interactions helping to optimize the cellular response to stimulation. One of the interferences includes the interaction between the store operated Ca^{2+} entry (SOCE) channel Orai1 with adenylyl cyclase 8 (AC8) that increase cAMP production, however its exact molecular mechanism is not known.
Aims & Methods: In this project we wanted to characterize the interactions of cAMP and Ca^{2+} signaling further focusing on the molecular components of SOCE. Human embrional kidney (HEK) cells were transfected with plasmids encoding the proteins of interest. Cellular cAMP production was measured by fluorescence resonance energy transfer (FRET) using the cAMP reporter Epac1.
Results: The stimulation of the cells with 5 μM forskolin and 100 μM 3-isobutyl-1-methylxanthine (IBMX) resulted in reversible elevation in cAMP production. The expression of AC8 significantly elevated the cAMP response. Whereas, Orai1 induced spontaneous cAMP production and a massive increase in the stimulated cAMP production. The effect of Orai1 was completely Ca^{2+} independent. Extended synaptotagmin 1 (E-Syt1), a recently described endoplasmic reticulum-plasma membrane tethering protein, increased the cAMP response, similarly to Orai1.
Conclusion: Our results showed that Orai1 and E-Syt1 play an important role in the regulation of cAMP production. However further studies are required to clarify the mechanisms of the interaction.
Disclosure of Interest: All authors have declared no conflicts of interest.

P0087 VX-809 RESTORES THE EXPRESSION DEFECT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR CAUSED BY ALCOHOL IN CAPAN-1 CELLS

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Introduction: Excessive ethanol consumption is one of the most common causes of acute and chronic pancreatitis. Earlier we showed that ethanol and ethanol metabolites cause severe damage in the function and expression of the cystic fibrosis transmembrane conductance regulator (CFTR), which increases the severity of acute ethanol-induced pancreatitis. There are new compounds available, such as lumacaftor (VX-809), to correct the impaired CFTR expression in cystic fibrosis patients, however the potential utility of this compound in pancreatitis treatment has never been investigated.
Aims & Methods: Our aim was to test the effect of VX-809 treatment on the CFTR expression during ethanol exposure. CFTR expression was evaluated by immunofluorescent staining in Capan-1 cells. The cells were incubated with 100 mM ethanol, 10 μM VX-809, or their combination for 24 h. Images were captured by confocal microscopy.
Results: As reported earlier exposure of Capan-1 cells to 100 mM ethanol for 24 hours significantly decreased the plasma membrane expression of CFTR. In parallel the cytoplasmic CFTR expression was increased. 10 μM VX-809 alone had no effect on the CFTR expression. Notably, application of 10μM VX-809 in pretreatment (treatment started 6 h prior to ethanol exposure), or post-treatment (treatment started 6 h after to ethanol exposure) significantly improved the plasma membrane expression of CFTR in Capan-1 cells.
Conclusion: These preliminary findings suggest that VX-809 might be able to restore the CFTR expression defect caused by alcohol. Further extended in vitro and in vivo studies need to clarify the effect of VX-809 on alcohol-induced pancreatic injury.
Disclosure of Interest: All authors have declared no conflicts of interest.