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Restoring CFTR function with Orkambi decreases the severity of alcohol-induced acute pancreatitis

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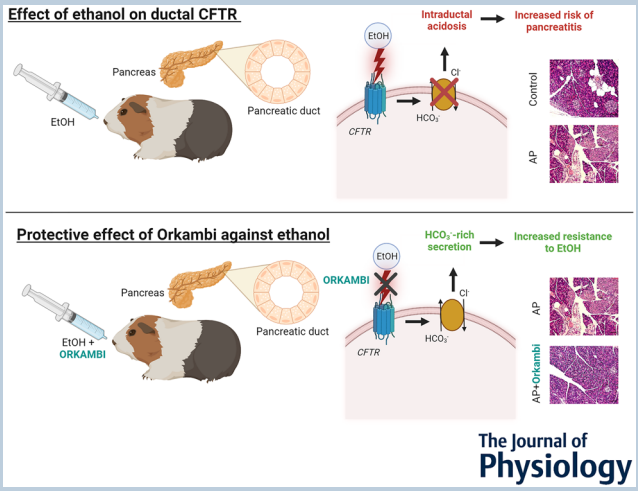

Abstract figure legend As a result of excessive alcohol consumption, the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel is severely damaged, which reduces pancreatic ductal HCO_3^- secretion. Decreased HCO_3^- secretion can lead to luminal acidosis, which can increase the risk of pancreatitis development. The CFTR modulator, Orkambi, restores normal CFTR function and thereby HCO_3^- secretion, which can reduce the severity of alcohol-induced acute pancreatitis (AP).

V. Venglovecz and A. Grassalkovich have contributed equally to this article.

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Abstract Heavy alcohol intake is one of the most common causes of acute pancreatitis (AP). We have previously shown that ethanol (EtOH) decreases the expression and activity of the cystic fibrosis transmembrane conductance regulator (CFTR), which plays a key role in alcohol-induced AP development. The prescription drug, Orkambi (a combination of ivacaftor and lumacaftor) can correct impaired CFTR function and expression in cystic fibrosis (CF) patients. Thus, the present study aimed to investigate whether Orkambi can mitigate alcohol-induced AP. Intact guinea-pig pancreatic ducts were pre-treated with different concentrations of ethanol (EtOH; 30, 50 and 100 mM) for 12 h alone or in combination with ivacaftor (VX770) and/or lumacaftor (VX-809), and CFTR expression and activity were evaluated by immunostaining and by the patch clamp technique, respectively. Alcoholic AP was induced in Orkambi-treated guinea-pigs, and standard laboratory and histological parameters were measured. Ivacaftor and lumacaftor alone or in combination dose-dependently restored the apical expression and activity of CFTR after EtOH treatment *in vitro*. Oral administration of Orkambi reduced the severity of alcohol-induced AP and restored impaired CFTR activity and expression. Orkambi is able to restore the CFTR defect caused by EtOH and decreases the severity of alcohol-induced pancreatitis. This is the first in vivo pre-clinical evidence of Orkambi efficacy in the treatment of alcohol-induced AP.

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Key points

- Acute pancreatitis is one of the leading causes of hospital admission among gastrointestinal diseases in which the lack of a specific drug therapy plays a crucial role.
- The cystic fibrosis transmembrane conductance regulator (CFTR) plays an essential role in pancreatic ductal HCO_3^- secretion; inappropriate CFTR function, as seen in heavy alcohol consumption, increases the risk of pancreatitis development.
- CFTR modulators are able to prevent the inhibitory effect of ethanol and reduce pancreatic ductal injury and the severity of alcohol-induced pancreatitis.
- CFTR modulators present a novel option in the pharmacotherapy of alcohol-induced pancreatitis by enhancing pancreatic functions or preventing recurrence.

Introduction

Acute pancreatitis (AP) is an inflammatory disease that affects 34 per 100 000 person-years in the general population (Parniczky et al., 2016; Petrov & Yadav, 2019). Although the disease is mostly mild, ∼20% of patients develop moderate or moderately severe pancreatitis, which can be life-threatening (Banks et al., 2013; Boxhoorn et al., 2020; Leppaniemi et al., 2019). Although therapy has improved a great deal in recent years, the deterioration of the patient's quality of life and the rate of mortality are still unacceptably high (Bang

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et al., 2020; Li et al., 2021; van Santvoort et al., 2011); it is therefore crucially important to identify new drug targets. One of the most important aetiological factors in the development of AP is excessive alcohol consumption, which is responsible for ∼30% of all cases in the USA (Sakorafas & Tsiotou, 2000; Wang et al., 2009; Weiss et al., 2019). Regular alcohol consumption increases the chance of AP recurrence and promotes the progression of AP to chronic pancreatitis (Bertilsson et al., 2015; Hegyi et al., 2021; Samokhvalov et al., 2015; Yadav & Lowenfels, 2013). The exact pathogenesis of alcohol-induced AP is only partly understood (Petersen et al., 2021), thus contributing to the lack of a specific therapy available for alcoholic AP patients.

Pancreatic ductal epithelial cells play an important role in the development and progression of AP (Hegyi, Maleth et al., 2011; Hegyi, Pandol et al., 2011; Judak et al., 2014; Maleth et al., 2015; Pallagi et al., 2011; Venglovecz et al., 2008; Venglovecz et al., 2018). The main function of pancreatic ductal epithelial cells is to secrete a HCO_3^--rich fluid, which neutralizes the acidic chyme in both the duodenum and the pancreas. In the duodenum, HCO_3^- provides suitable pH conditions for the proper function of digestive enzymes, whereas it prevents the harmful effects of acid in the pancreas (Hegyi, Maleth et al., 2011). The cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel plays an important role in ductal HCO_3^- secretion (Park et al., 2010). CFTR is located on the apical membrane of ductal cells, where it works in close interaction with the SLC26a6 Cl^-/HCO_3^- exchanger (Shcheynikov et al., 2006). Improper functioning of CFTR, as seen in cystic fibrosis (CF), reduces ductal fluid and HCO_3^- secretion, which can increase the risk of pancreatitis development (Hegyi & Petersen, 2013; Maleth et al., 2015; Pallagi et al., 2014, 2015). We have previously shown that the activity and membrane expression of CFTR is significantly decreased under the influence of ethanol (EtOH) (Judak et al., 2014; Maleth et al., 2015). High doses of EtOH strongly damaged ductal mitochondria, leading to ATP depletion and lowered CFTR activity. In addition, EtOH caused inadequate folding and trafficking of CFTR, resulting in reduced expression of the channel at the apical membrane. Decreased CFTR function results in reduced ductal HCO_3^- and fluid secretion, thus causing intraductal acidosis, which accelerates trypsinogen autoactivation and leads to pancreatitis. These results are consistent with previous observations that CF patients are at high risk of developing pancreatitis (Hegyi et al., 2016; Noone et al., 2001; Ooi et al., 2011). In addition, Fur et al. (2021) have shown that there is CFTR mislocalization in cerulein-induced acute pancreatitis, in which VX-661 + VX-770 treatment has beneficial effects.

Orkambi is a clinically available CFTR modulator that has been developed by Vertex Pharmaceuticals (Boston, MA, USA) and is used effectively in the therapy of CF patients, especially in those patients who are homozygous for the F508del mutation (Favia et al., 2020). Orkambi contains both a CFTR potentiator (ivacaftor or VX-770) and a corrector (lumacaftor or VX-809). The potentiator directly binds to the channel and increases its open probability, whereas the corrector acts as a pharmacological chaperone that improves the folding of the CFTR protein and thus its trafficking to the apical plasma membrane (Eckford et al., 2014; Ren et al., 2013; van Goor et al., 2009, 2011). A beneficial effect of ivacaftor on AP has been suggested in clinical studies (Carrion et al., 2018; Johns & Rowe, 2019; Kounis et al., 2018). Carrion et al. (2018) found that 9 months of ivacaftor therapy eliminated AP recurrence in CF patients. In another study, ivacaftor treatment improved the pancreatic manifestations of a CF patient (Kounis et al., 2018). As a result of the treatment, the patient's faecal elastase level normalized, the need for pancreatic enzyme replacement drastically dropped and AP did not recur during the therapy. Johns & Rowe (2019) also suggested that Ivacaftor could be a promising drug in the treatment of recurrent pancreatitis in CF patients.

Therefore, the present study aimed to test the active substances in Orkambi in alcohol-induced CFTR damage and to investigate the effect of orally-administered Orkambi in alcohol-induced pancreatitis.

Methods

Chemicals and solutions

All general laboratory chemicals were purchased from Sigma-Aldrich (Budapest, Hungary). Cryomatrix was from Thermo Scientific (Waltham, MA, USA). CLSPA collagenase was obtained from Worthington Biochemical Corporation (Lakewood, NJ, USA). Cerulein was from the American Peptide Company (Sunnyvale, CA, USA).

Animals

Guinea-pigs (4–12 weeks old, both sexes) were kept in standard plastic cages under a 12:12 h light/dark photocycle at room temperature (23 ± 1°C) with free access to standard laboratory chow and water. Animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (United States, Department of Health and Human Services). In addition, the experimental protocol was approved by the local Ethical Board at the University of Szeged, Hungary (KA-3436).

Isolation and treatment of the ducts

Intra-interlobular ducts were isolated from the pancreas of guinea-pigs that weighed 250–350 g. The guinea-pig was humanely killed by cervical dislocation, the pancreas was removed and intra-interlobular ducts were isolated, as described previously (Venglovecz et al., 2008). The ducts were cultured overnight in an incubator at 37°C gassed with 5% CO_2/95% air. After the incubation, pancreatic ducts were treated with EtOH (30, 50 and 100 mM) for 12 h with or without the administration of VX-770 (1, 3, 5 and 10 μM), VX-809 (1, 3, 5 and 10 μM) or a combination of VX-770 and VX-809 (1, 3, 5 and 10 μM) for 2, 5 or 10 h.

Immunofluorescence staining and quantification

After the treatments, pancreatic ducts were embedded into cryomatrix, and then frozen sections (7 μm) were prepared with a cryostat (Leica, Wetzlar, Germany). The frozen sections were fixed in 4% paraformaldehyde for 10 min, washed three times with Tris-buffered saline and then permeabilized with 0.1% Triton X-100 for 10 min at room temperature. After being blocked with 1% normal goat serum for 60 min, the sections were incubated with anti-CFTR rabbit polyclonal IgG antibody (catalog. no. ACL-006; Alomone Labs, Jerusalem, Israel) overnight at 4°C. On the next day, sections were incubated with Alexa Fluor 488 dye-conjugated secondary antibody (Molecular Probes, Waltham, MA, USA) for 2 h at room temperature in the dark. Nuclei were counterstained with Hoechst 33342 (Thermo Fisher Scientific, Darmstadt, Germany). Slices were mounted using Fluoromount, and pictures were taken using a LSM 880 confocal laser scanning microscope (Carl Zeiss Technika Kft., Budaörs, Hungary) with a 40× objective lens (Plan-Apochromat 40×/1.4 oil). Secondary antibody (Alexa Fluor 488) and Hoechst 33342 were excited at 488 and 405 nm and detected at 405–465 nm (Hoechst 33342) and 488–550 nm (CFTR), respectively. The intensity was given in fluorescence units (AU). CFTR expression was measured using ImageJ (NIH, Bethesda, MD, USA) in 10 cells per sample separately in the apical membrane and in the cytoplasm. Then, based on the quantified CFTR intensities, statistical tests (two-sample *t* test and ANOVA) were performed.

Measurements of CFTR activity

The whole cell configuration of the patch clamp technique was used to measure CFTR channel activity. Intact pancreatic ducts were treated with EtOH (30 mM) with or without VX-770 (10 μM), VX-809 (10 μM) or a combination of VX-770 and VX-809 (10 μM) for 12 h. Control groups had the same treatment without EtOH. After the treatments, single pancreatic ductal cells were isolated from the intact ducts by an enzymatic micro-dissection procedure, as previously described (Hegyi, Maleth et al., 2011). Patch clamp micropipettes were pulled from borosilicate glass capillaries (GB150F-8P; Science Products GmbH, Hofheim, Germany) using a P-97 Flaming/Brown micropipette puller (Sutter, Novato, CA, USA) with resistances between 3.5 and 6.5 MΩ. Whole cell currents were recorded with a HEKA EPC 10 patch clamp amplifier (Warner Instruments, Holliston, MA, USA) at room temperature. The standard extracellular solution contained (in mM): 145 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes and 5 glucose (pH 7.4 with NaOH). The osmolarity of the external solution was 300 mosmol L^{-1}. The standard pipette solution contained (in mM): 120 CsCl, 2 $MgCl_2$, 10 HEPES, 5 EGTA and 1 Na_2ATP (pH 7.2 with CsCl). The osmolarity of the pipette solution was 240 mosmol L^{-1}. After establishing a high (1–10 GΩ) resistance seal by gentle suction, the cell membrane was disrupted by further suction or by applying a 1.5 V electrical pulse for 1–5 ms. The membrane potential (Vm) was held at 0 mV and then clamped to ±100 mV in 20 mV increments for 500 ms with an 800 ms interval between each pulse to obtain current–voltage (*I–V*) relationships. Currents were measured over a 4 ms period starting at 495 ms into the voltage pulse. Mean current amplitudes were calculated at ±60 mV and normalized to cell capacitance (pF).

Induction of acute pancreatitis

Because there is no accepted protocol for the induction of alcoholic AP in guinea-pigs, this was set up as a first step. Guinea-pigs (200–250 g) were randomly allocated into four groups ($n = 5$) as follows: (1) control; (2) high-fat diet + 2.5% (vol/vol) EtOH *ad libitum*; (3) the same as group 2 but supplemented with 25% EtOH via oral gavage every 3 days; and (4) the same as group 3 but supplemented with 750 mg kg^{-1} palmitoleic acid and 1.75 g kg^{-1} EtOH I.P. every 3 days. In case of the high-fat diet, the normal rodent chow was supplemented with 5% coconut fat, 2.5% lard and 0.15% cholesterol, based on data in the literature (Arias-Mutis et al., 2017, 2018). To accustom the animals to the high-fat diet, they were fed a mixture of 50% normal and 50% high-fat diet for 1 week before the treatments. Oral gavage of EtOH was performed with a plastic tube (13 gauge × 150 mm; Instech Laboratories, Plymouth Meeting, PA, USA) as previously described (Toth et al., 2019). Treatments lasted for 18 days, the animals were then killed on the following day by pentobarbital overdose (200 mg/kg I.P.), and histological and laboratory parameters were examined. Protocols are illustrated in Fig. 1.

Treatment with Orkambi

Guinea-pigs were treated orally using a plastic tube with 7.143 mg kg^{-1} VX-770 and 8.929 mg kg^{-1} VX-809 every third day for 18 days. Concentrations of VX-770 and VX-809 were calculated from the drug doses used in humans. The oral treatment was carried out with a plastic probe. VX-770 and VX-809 were dissolved in physiological saline containing 8.3% polyoxyl 40 hydrogenated castor oil and 8.3% ethanol (Rehman et al., 2011).

Histological examination and laboratory parameter measurements

After the terminal anaesthesia, the abdominal and thoracic cavities were opened with a median laparotomy and thoracotomy and then 3 mL of blood was collected with cardiac puncture. The collected blood samples were transferred to Eppendorf tubes and were centrifuged at 2500 × *g* for 20 min at 4°C, and then the serum was stored at –20°C until use. Serum amylase activity was measured with a commercial colorimetric kit with a FLUOstar OPTIMA (BMG Labtech, Budapest, Hungary) microplate reader at 405 nm.

The pancreas was removed and trimmed from fat and lymphatic tissues on ice for the histological examination. The pancreatic head and body were longitudinally dissected and placed in 6% formaldehyde solution. After fixation, pancreas samples were embedded in paraffin and then cut into 3 μm thick slices and stained with haematoxylin and eosin. Oedema, necrosis and leukocyte infiltration were classified using a semi-quantitative system. Oedema was graded from 0 to 3 (0 = none; 1 = local interlobular; 2 = diffuse interlobular; 3 = diffuse interlobular and intraacinar) and leukocyte infiltration was graded from 0 to 3 (0 = none; 1 = local interlobular; 2 = diffuse interlobular; 3 = diffuse interlobular and intraacinar). The degree of necrosis was given as a percentage (Kui et al., 2015; Toth et al., 2019).

Ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS)

Serum samples were measured with UHPLC-MS/MS in accordance with a previously published methodology (Galla et al., 2021). The multiple reaction monitoring

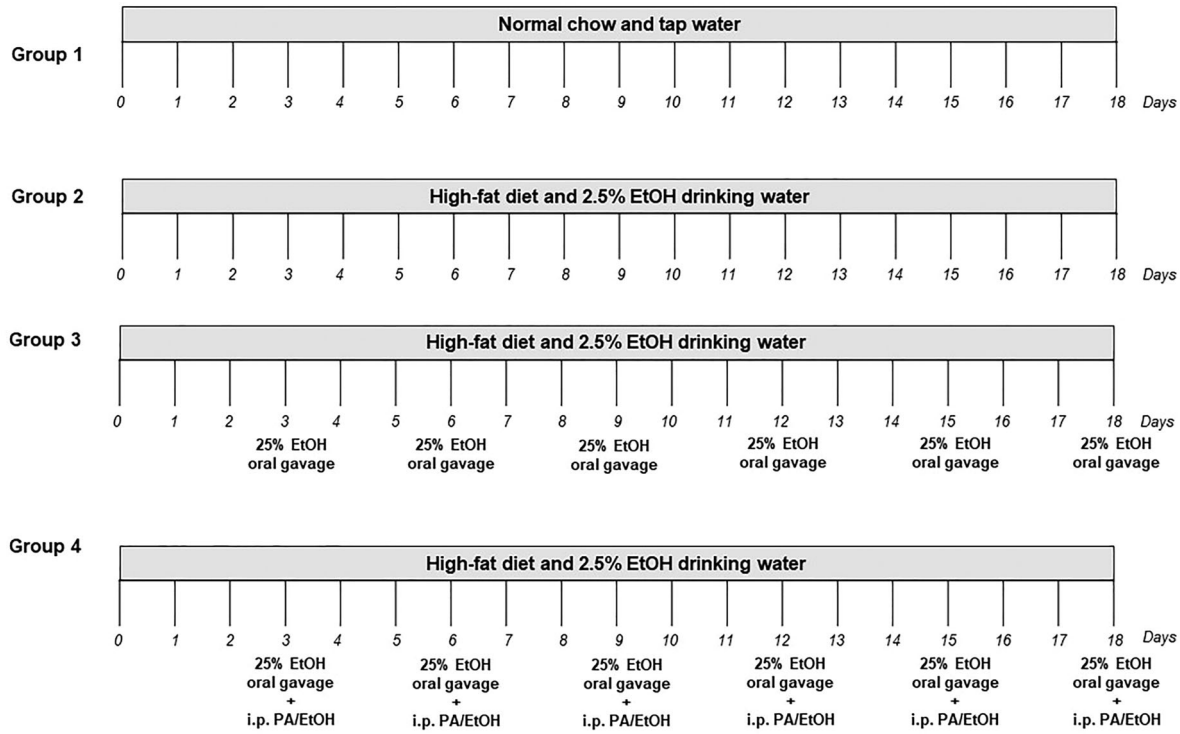

Figure 1. Protocols for the induction of acute pancreatitis in guinea-pigs
The schematic diagram shows the treatment protocols. Guinea-pigs (200–250 g, both sexes) were randomly allocated into four groups (n = 5) as follows: group 1: the animals were kept on standard chow and tap water; group 2: the animals were kept on a high-fat diet, and drinking water was supplemented with 2.5% (vol/vol) ethanol (EtOH) *ad libitum*; group 3: the animals were treated the same as those in group 2, supplemented with 25% EtOH via oral gavage every 3 days; and group 4: the animals were treated the same as those in group 3, supplemented with 750 mg kg^{-1} palmitoleic acid (PA) and 1.75 g kg^{-1} EtOH I.P. every 3 days.

transition of ivacaftor was 393.1/172.1 using 100 V as declustering potential and 25 V as collision energy, with a retention time of 10.51 min. The multiple reaction monitoring transition of lumacaftor was 453.1/103.0 using 145 V as declustering potential and 82 V as collision energy, with a retention time of 10.65 min.

Enzyme-linked immunosrbent assay (ELISA)

Serum levels of interleukin-1β (IL-1β) were determined using an ELISA kit (catalog. no. EGP0029; FineTest Biotech Co., Ltd, Wuhan, China) in accordance with the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed with Prism, version 10.3.0 (GraphPad Software Inc., San Diego, CA, USA). Data are expressed as the mean ± SD. The Shapiro–Wilk test was used to analyse data distribution. A significant difference between groups was determined by one-way ANOVA, followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

Results

VX-770 and VX-809 prevent the EtOH-induced decrease in CFTR expression

Intact pancreatic ducts were incubated with different concentrations of EtOH (30, 50 and 100 mM) for 12 h and the expression of CFTR was investigated by immunohistochemistry (Fig. 2). Strong CFTR expression was detected on the apical membrane of the isolated duct, as expected. In response to EtOH treatments, a significant decrease in CFTR expression was observed, and the histological structure of the ducts were also impaired (Fig. 2*A*). The continuity of the epithelial layer was broken

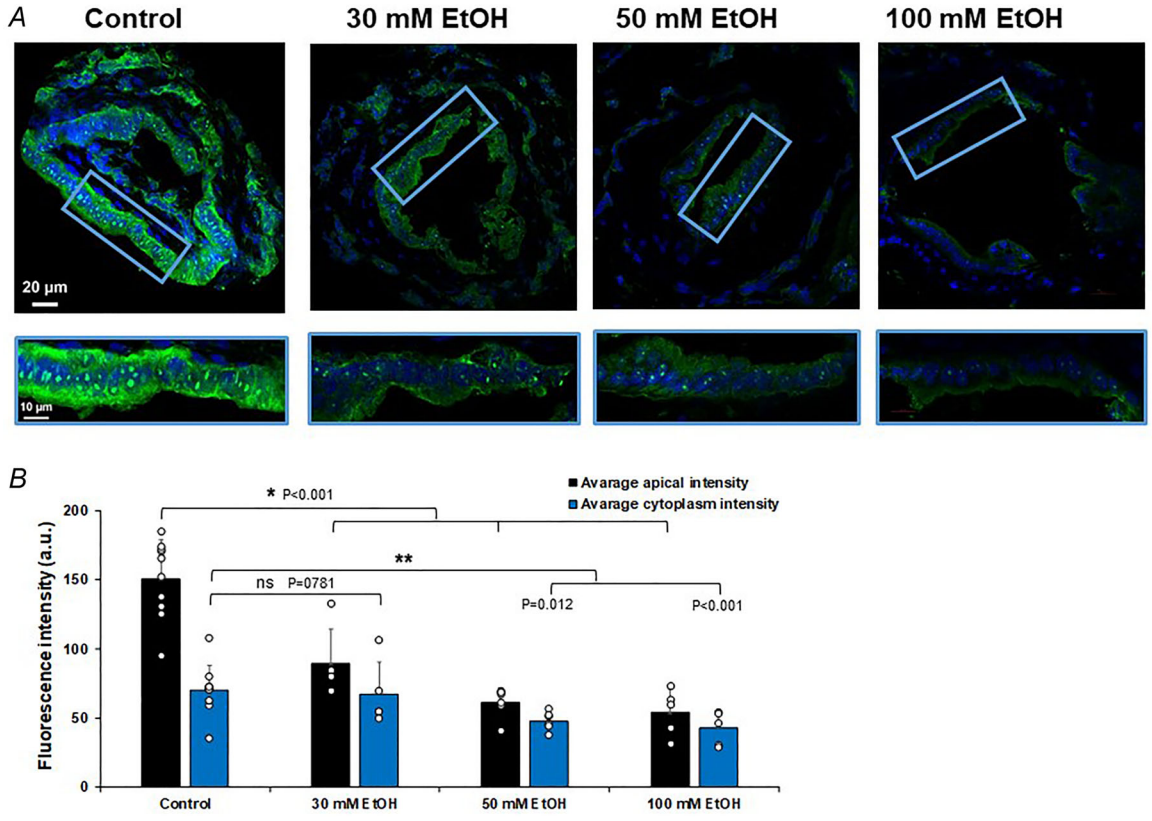

Figure 2. Effect of ethanol on CFTR expression
A, representative histological images show CFTR expression after treatment of the pancreatic ducts with ethanol (EtOH; 30, 50 and 100 mM) for 12 h. Pancreas slices were excited at 405 nm (Hoechst 33342) and 488 nm (Alexa Fluor 488), and emissions were collected at 405–465 and 488–550 nm, respectively. *B*, summary bar charts show the mean fluorescence intensity in the ductal cells normalized to the ductal area and expressed in arbitrary units. Data are presented as the mean ± SD. *$P < 0.001$ (30 mM EtOH), $P < 0.001$ (50 mM EtOH) and $P < 0.001$ (100 mM EtOH) *vs*. apical staining intensity of control. **$P = 0.012$ (50 mM EtOH) and $P < 0.001$ (100 mM EtOH) *vs*. cytoplasm staining intensity of control. ns = no significance, $P = 0.781$ (30 mM EtOH *vs*. cytoplasm staining intensity of control) (n = 5–8 per group).

in many cases, the volume of the ductal cells decreased and cytoplasmic protrusions were observed. CFTR expression was examined separately in the apical membrane and in the cytoplasm (Fig. 2*B*). In the apical membrane, a dose-dependent drop was observed [150 ± 28.08 AU *vs.* 89.6 ± 24.8 AU (30 mM EtOH, $P < 0.001$), 61.1 ± 10.8 AU (50 mM EtOH, $P < 0.001$) and 54 ± 16.8 AU (100 mM EtOH, $P < 0.001$)] (Fig. 2*B*). By contrast, only higher doses of EtOH decreased channel expression in the cytoplasm [70.2 ± 18 AU *vs.* 67.1 ± 23.1 AU (30 mM EtOH, $P = 0.781$), 47.9 ± 6.8 AU (50 mM EtOH, $P = 0.012$) and 42.7 ± 12.2 AU (100 mM EtOH, $P < 0.001$)].

Next, we investigated the effect of the CFTR corrector (VX-809) and activator (VX-770) on the EtOH-induced reduced CFTR expression. The active substances alone did not significantly affect the expression of CFTR (data not shown, $n = 5$ per group). Isolated pancreatic ducts were incubated with EtOH (30 mM) and different concentrations of VX-809 (1, 3, 5 and 10 μM) for 12 h, and then the expression of CFTR was investigated. As shown in Fig. 3, VX-809 was able to prevent the inhibitory effect of EtOH even at the lowest concentration (1 μM) (Fig. 3*A* and *B*). Next, we examined the time-dependent effect of the corrector. Isolated pancreatic ducts were incubated with EtOH (30 mM) for 12 h, during which the culture media was supplemented with 10 μM VX-809 at different time points (3, 7 and 10 h after the initiation of EtOH treatment). The corrector was able to restore the expression of CFTR even after 2 h of incubation, whereas no further increase in the expression was observed with longer incubation times (5 and 10 h) (Fig. 3*C* and *D*). The corrector did not significantly affect the cytoplasmic expression of CFTR at any concentration or incubation time (Fig. 3*B* and *D*).

These treatment protocols were also carried out for the activator (VX-770) (Fig. 4. By contrast to VX-809, we found that only the highest concentrations (5 and 10 μM) of VX-770 were able to restore CFTR expression after EtOH treatment [$P = 0.079$ (5 μM) and $P = 0.411$ (10 μM) *vs.* control], whereas, similarly to VX-809, VX-770 did not affect CFTR expression in the cytoplasm. In terms of time dependence, we found that at least 10 h of VX-770 treatment is necessary to counteract the inhibitory effect of EtOH ($P = 0.825$ *vs.* control) (Fig. 4*C* and *D*).

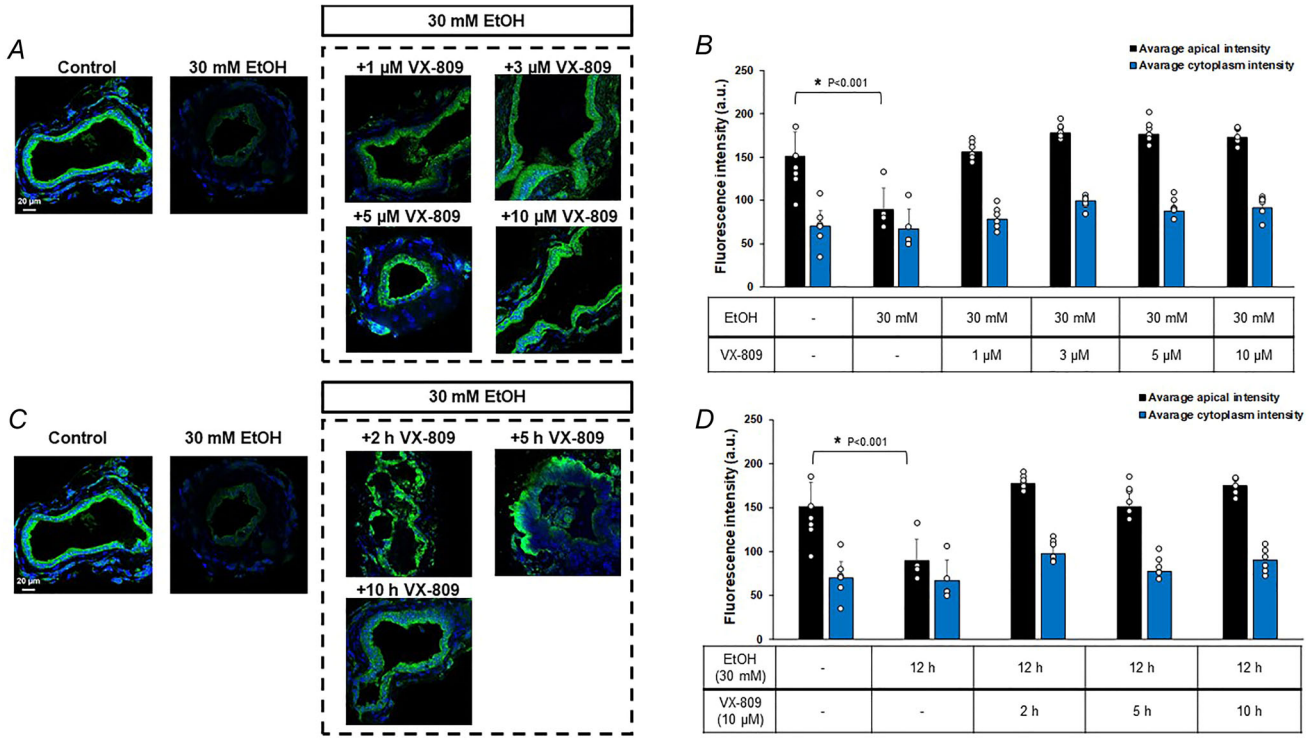

Figure 3. Effect of VX-809 on CFTR expression
Representative histological images show CFTR expression after treatment of the pancreatic ducts with ethanol (EtOH; 30 mM) and VX-809 (1, 3, 5 and 10 μM) for 12 h (*A*) or VX-809 (10 μM) for 2, 5 and 10 h (*C*). Pancreas slices were excited at 405 nm (Hoechst 33342) and 488 nm (Alexa Fluor 488), and emissions were collected at 405–465 and 488–550 nm, respectively. Scale bar = 20 μm. Summary bar charts show the dose-dependent (*B*) and time-dependent (*D*) effect of VX-809. Mean fluorescence intensity in the ductal cells was normalized to the ductal area and expressed in arbitrary units. Data are presented as the mean ± SD. *$P < 0.001$ (30 mM EtOH) *vs.* apical staining intensity of control (n = 5–8 per group).

We also examined the effect of a combination of the two drugs (Fig. 5). We incubated the ducts with EtOH (30 mM) and examined a combination of VX-809 and VX-770 at various concentrations (1, 3, 5 and 10 μM) for 12 h, as well as CFTR expression. As shown in Fig. 5*A* and *B*, the combination treatment restored CFTR expression even at the lowest concentration (1 μM) ($P = 0.843$ *vs.* control). We also examined the effect of the combination treatment at higher EtOH concentrations (Fig. 5*C* and *D*). A 10 μM combination treatment was able to prevent the CFTR-damaging effect of 50 mM EtOH but was not effective in case of 100 mM EtOH in the case of apical expression of CFTR [$P = 0.884$ (50 mM EtOH + 10 μM combination) and $P < 0.001$ (100 mM EtOH + 10 μM combination) *vs.* control]. However, CFTR cytoplasmic expression was completely restored by the combination treatment at both EtOH concentrations [$P = 0.737$ (50 mM EtOH + 10 μM combination) and $P = 0.658$ (100 mM EtOH + 10 μM combination) *vs.* control].

VX-770 and VX-809 prevent the EtOH-induced decrease in CFTR activity

In the next step, we investigated the effects of EtOH (30 mM), VX-809 (10 μM), VX-770 (10 μM) and a combination of VX-809 and VX-770 (10 μM) on the activity of CFTR using the whole cell configuration of the patch clamp technique (Fig. 6). Because CFTR channel activity is very low under resting conditions, the effects of EtOH and CFTR modulators were investigated on forskolin-stimulated currents. Ducts were pre-treated with EtOH alone or in combination with VX-809 and/or VX-770 for 12 h. Then individual ductal cells were isolated, and the activity of CFTR was measured. EtOH treatment significantly reduced the forskolin-stimulated CFTR currents compared to the control (139.3 ± 10.5 *vs.* 51.4 ± 30 pA/pF at 60 mV; $P < 0.001$ *vs.* control). VX-809 alone did not significantly affect the magnitude of the CFTR currents, whereas treatment with VX-770 significantly increased

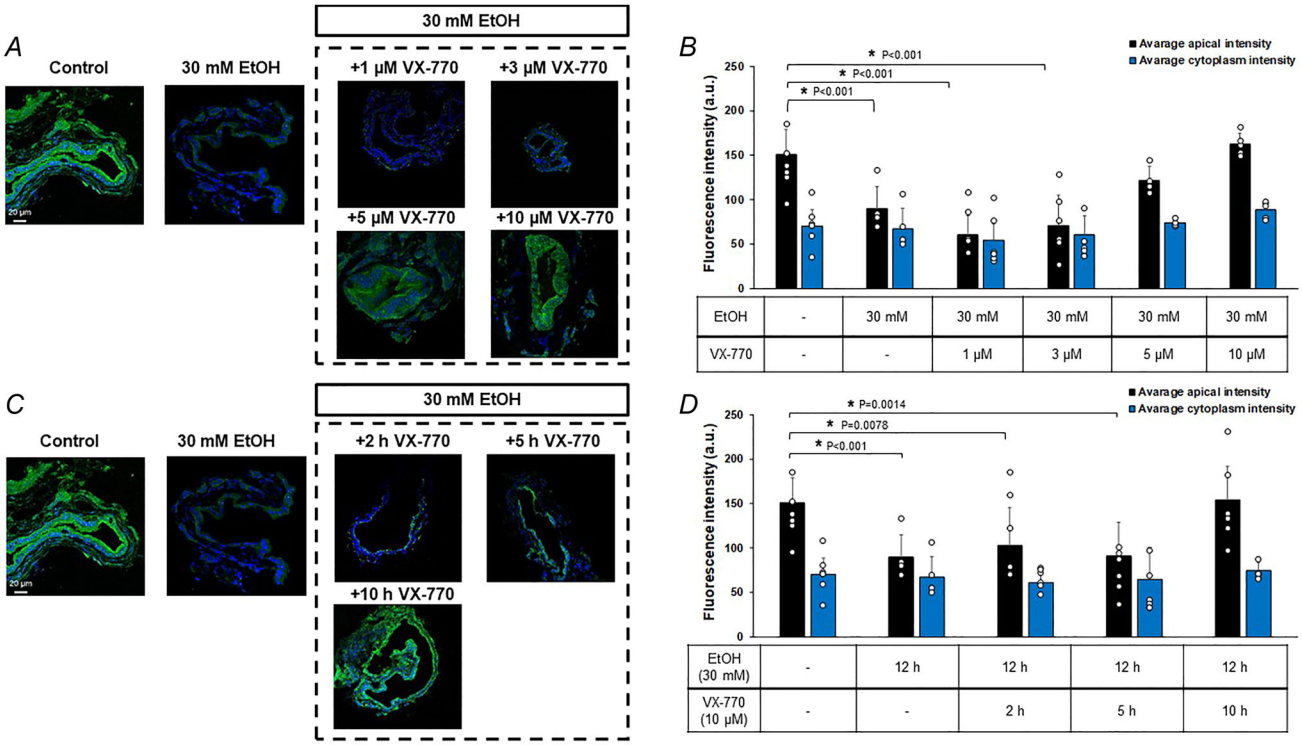

Figure 4. Effect of VX-770 on CFTR expression
Representative histological images show CFTR expression after treatment of the pancreatic ducts with ethanol (EtOH; 30 mM) and VX-770 (1, 3, 5 and 10 μM) for 12 h (*A*) or VX-770 (10 μM) for 2, 5 and 10 h (*C*). Pancreas slices were excited at 405 nm (Hoechst 33342) and 488 nm (Alexa Fluor 488), and emissions were collected at 405–465 and 488–550 nm, respectively. Scale bar = 20 μm. Summary bar charts show the dose-dependent (*B*) and time-dependent (*D*) effect of VX-809. Mean fluorescence intensity in the ductal cells was normalized to the ductal area and expressed in arbitrary units. Data are presented as the mean ± SD. *$P < 0.001$ (30 mM EtOH), $P < 0.001$ (1 μM VX-770), $P < 0.001$ (3 μM VX-770), $P = 0.0078$ (2 h VX-770) and $P = 0.0014$ (5 h VX-770) *vs.* apical staining intensity of control (n = 5–8 per group).

it (448.2 ± 79.7 pA/pF at 60 mV; $P < 0.001$ *vs*. control). Interestingly, coadministration of the two agents (10 μM) caused a much greater increase in CFTR activity than VX-770 alone (613.4 ± 77.3 pA/pF *vs*. 448.2 ± 79.7 pA/pF at 60 mV; $P = 0.024$ *vs*. VX-770), indicating that the two agents potentiate each other's effects. Both VX-770 and VX-809 were able to prevent the EtOH-induced drop in CFTR activity [237 ± 32.9 and 190.5 ± 26.2 pA/pF *vs*. 51.4 ± 30 pA/pF at 60 mV; $P < 0.001$ (VX-770 + EtOH) and $P < 0.001$ (VX-809 + EtOH) *vs*. EtOH] and this protective effect was also observed with the combination treatment (562.9 ± 113.5 pA/pF at 60 mV; $P < 0.001$ (combination + EtOH) *vs*. EtOH).

Orkambi decreases the severity of acute pancreatitis

To investigate the protective effect of Orkambi under *in vivo* conditions, we investigated the effect of Orkambi on the course of AP. There is no accepted protocol for the induction of AP in guinea-pigs; therefore, as a first step, we worked out an AP model (Figs 1 and 7).

Guinea-pigs were randomly allocated into four groups ($n = 5$) as described in the Methods. A high-fat diet combined with alcoholic drinking water (group 2) caused no significant changes in the structure of the pancreas (Fig. 7*A*). Based on the histological image, mild, oedematous pancreatitis developed without obvious signs of necrosis. When the above protocol was supplemented with the forced drinking of a high concentration of alcohol every 3 days (group 3), tissue edge damage, increased fat deposition and vacuolization were observed. In group 4, where the treatment was supplemented with I.P. EtOH/fatty acid injections every 3 days, moderate to severe interstitial oedema was observed with extensive infiltration of leukocytes. Furthermore, I.P. injection of EtOH/palmitoleic acid caused large necrotic areas in the pancreas of some animals. The extent of oedema, leukocyte infiltration and necrosis can be seen in Fig. 7*B*–*D*. Interestingly, although the serum amylase

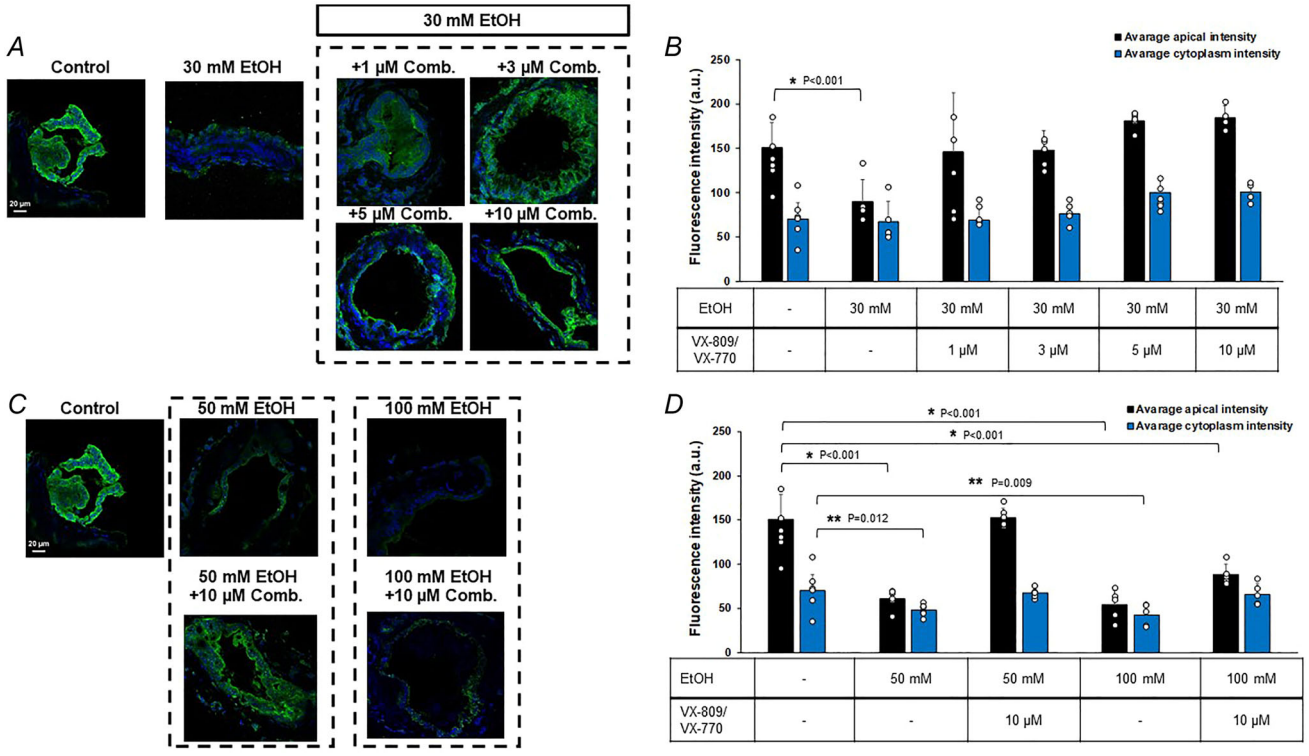

Figure 5. Effect of VX-809/VX-770 combination treatment on CFTR expression.
Representative histological images show CFTR expression after treatment of the pancreatic ducts with ethanol (EtOH; 30 mM) and a combination of VX-809 and VX-770 (1, 3, 5 and 10 μM) (*A*) or with different concentrations of EtOH (50 and 100 mM) and a combination of VX-809 and VX-770 (10 μM) (*C*). Pancreas slices were excited at 405 nm (Hoechst 33342) and 488 nm (Alexa Fluor 488), and emissions were collected at 405–465 and 488–550 nm, respectively. Scale bar = 20 μm. Summary bar charts show the effect of the combination at 30 mM (*B*) and at 50 and 100 mM (*D*) EtOH. Mean fluorescence intensity in the ductal cells was normalized to the ductal area and expressed in arbitrary units. Data are presented as the mean ± SD. *$P < 0.001$ (30 mM EtOH), $P < 0.001$ (50 mM EtOH), $P < 0.001$ (100 mM EtOH) and $P < 0.001$ (100 mM EtOH + 10 μM combination) *vs*. apical staining intensity of control. $P = 0.012$ (50 mM EtOH) and $P = 0.009$ (100 mM EtOH) *vs*. cytoplasm staining intensity of control (n = 5–8 per group).

activity slightly increased in groups 3 and 4, this was not significant compared to the control [$P = 0.093$ (group 3) and $P = 0.061$ (group 4) *vs.* control] (Fig. 7*E*). Overall, we found that both groups 3 and 4 developed moderate to severe pancreatitis as a result of the treatments. Because the mortality rate was higher in group 4 (two out of five), protocol 3 was used to study the *in vivo* effect of Orkambi.

Orkambi treatment was administered orally in a single dose 1 h after each oral EtOH gavage (see Methods). Blood was taken from the control and EtOH-treated animals and the concentrations of VX-770 and VX-809 were analysed by HPLC to determine whether Orkambi was absorbed in sufficient quantities after oral administration (Fig. 8*A*). Neither drug was detected in animals not treated with Orkambi. By contrast, both VX-770 and VX-809 were present in the serum of the Orkambi-treated control (VX-770: 3244.3 ± 904.1 nM and VX-809: 11 287.9 ± 6276.7 nM) and AP (VX-770: 3321.8 ± 1700.6 nM and VX-809: 16 145.7 ± 6482.2 nM) animals. Serum amylase activity did not change in either the AP or Orkambi-treated groups compared to the control (Fig. 8*B*). The extents of pancreatic oedema and tissue necrosis were characterized to determine the rate of pancreatic damage (Fig. 8*C* and *D*). High-fat/EtOH treatment caused markedly elevated acinar cell necrosis (13.1 ± 5.5%) and interstitial oedema (1.4 ± 0.5), which were significantly ameliorated as a result of the Orkambi treatment (oedema score: 0.6 ± 0.5 *vs.* 1.4 ± 0.5, $P = 0.049$; necrosis: 6.3 ± 4.9% *vs.* 13.1 ± 5.5%; $P = 0.015$). By contrast, oral administration of Orkambi alone did not cause significant oedema or acinar cell necrosis (oedema score: 0.3 ± 0.4 *vs.* 0.6 ± 0.5, $P = 0.37$; necrosis: 4 ± 2.2% *vs.* 4.4 ± 0.89, $P = 0.72$). In the case of leukocyte infiltration, no significant changes were observed in either the AP or Orkambi-treated groups (Fig. 8*E*). Representative histological images (Fig. 8*F*) show that the extent of EtOH-induced severe pancreatic necrosis was significantly reduced by oral Orkambi treatment. Furthermore, the effect of Orkambi on the serum IL-1β concentration was also examined. Orkambi alone slightly lowered levels of IL-1β in the control animals. In the AP group, its level significantly increased compared to the control (38.9 ± 7.6 pg mL^{-1} *vs.* 23.7±6.7 pg mL^{-1}, $P = 0.024$) (Fig. 8*G*) and it was significantly reduced by oral Orkambi treatment (16 ± 2.8 pg mL^{-1}, $P < 0.001$).

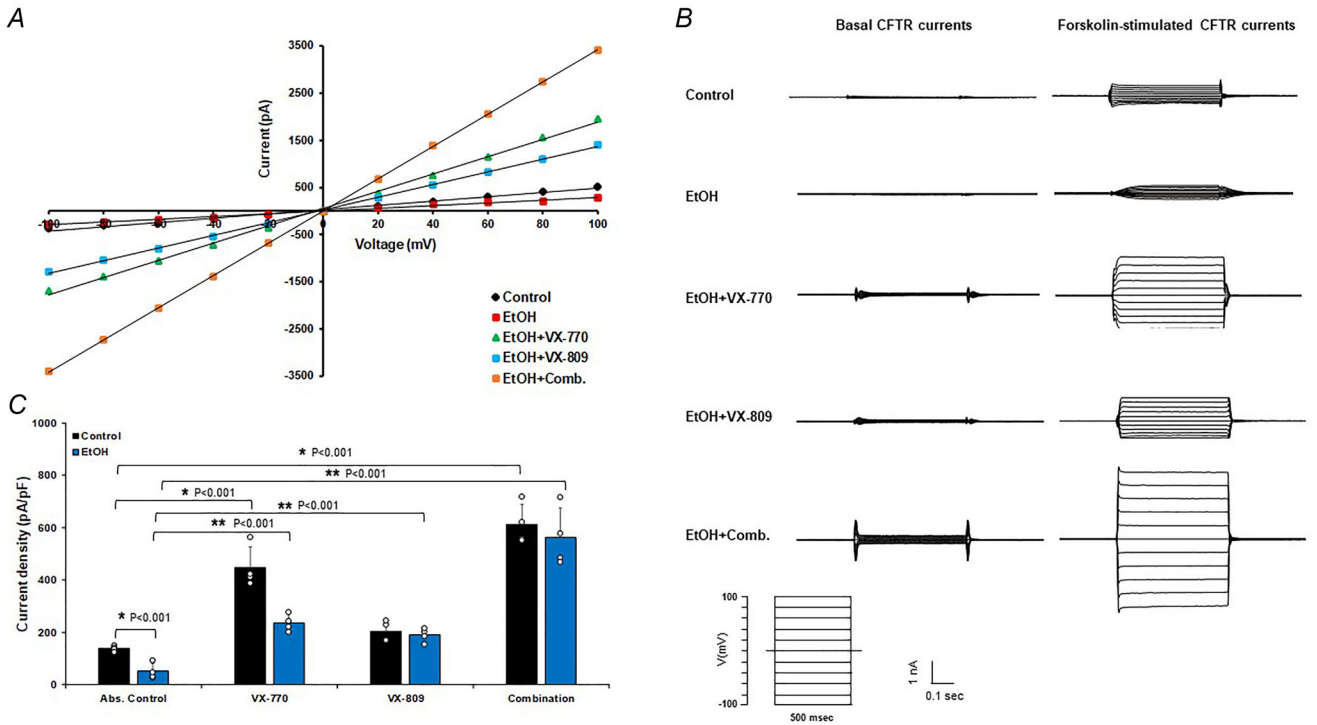

Figure 6. Effect of ethanol and CFTR modulators on the activity of CFTR
A, current–voltage (*I–V*) relationships were obtained by measuring forskolin-stimulated CFTR currents over a 4 ms period starting 495 ms into the voltage pulse. *B*, representative whole cell currents recorded before and after stimulation with 5 μM forskolin. *C*, summary of current densities (measured at +60 mV) in forskolin-stimulated cells. The current densities have been normalized to the cell input capacitance (pA/pF). Data are presented as the mean ± SD. **P* < 0.001 (30 mM EtOH), *P* < 0.001 (10 μM VX-770) and *P* < 0.001 (10 μM combination) *vs.* control. ***P* < 0.001 (10 μM VX-770 + 30 mM EtOH), *P* < 0.001 (10 μM VX-809 + 30 mM EtOH) and *P* < 0.001 (10 μM combination + 30 mM EtOH) *vs.* ethanol (*n* = 3–5 per group).

Orkambi restores activity and expression of CFTR in acute pancreatitis

Treatment of guinea-pigs with Orkambi for 18 days increased the activity of the CFTR channel compared to the control (283 ± 37.8 *vs.* 139.3 ± 10.5 pA/pF at 60 mV, $P < 0.001$) (Fig. 9*A* and *B*). Induction of AP significantly lowered the activity of the channel, which was barely detected (4.4 ± 4.7 pA/pF at 60 mV, $P < 0.001$). Conversely, in animals where AP induction was supplemented with oral Orkambi treatment, channel activation returned to the control value (124.6 ± 22.9 pA/pF at 60 mV, $P < 0.001$). We have also used immunohistochemistry to investigate the effect of AP on CFTR expression. Strong CFTR staining was detected in the control group, especially on the apical membrane of the ductal cells (Fig. 9*C* and *D*). CFTR staining was more intense as a result of oral Orkambi treatment, in both the apical membrane and the cytoplasm. EtOH-induced AP significantly reduced CFTR expression in the apical plasma membrane, which was completely restored by Orkambi treatment ($P = 0.028$ *vs.* AP) (Fig. 9*C* and *D*).

Discussion

Excessive alcohol consumption is one of the main aetiological factors in the development of AP (Bertilsson et al., 2017; Samokhvalov et al., 2015). The therapy of alcoholic AP is mainly supportive because there is currently no specific pharmacotherapy that would be effective. Despite intensive research, the mechanism by which alcohol predisposes to the development of pancreatitis is still not fully understood, thus partially explaining the lack of specific therapy. In the present study, we investigated the impacts of the CFTR modulator Orkambi on the severity of alcohol-induced AP. We provided the first *in vivo* evidence that Orkambi decreases the severity of experimental pancreatitis by preventing EtOH-induced CFTR damage.

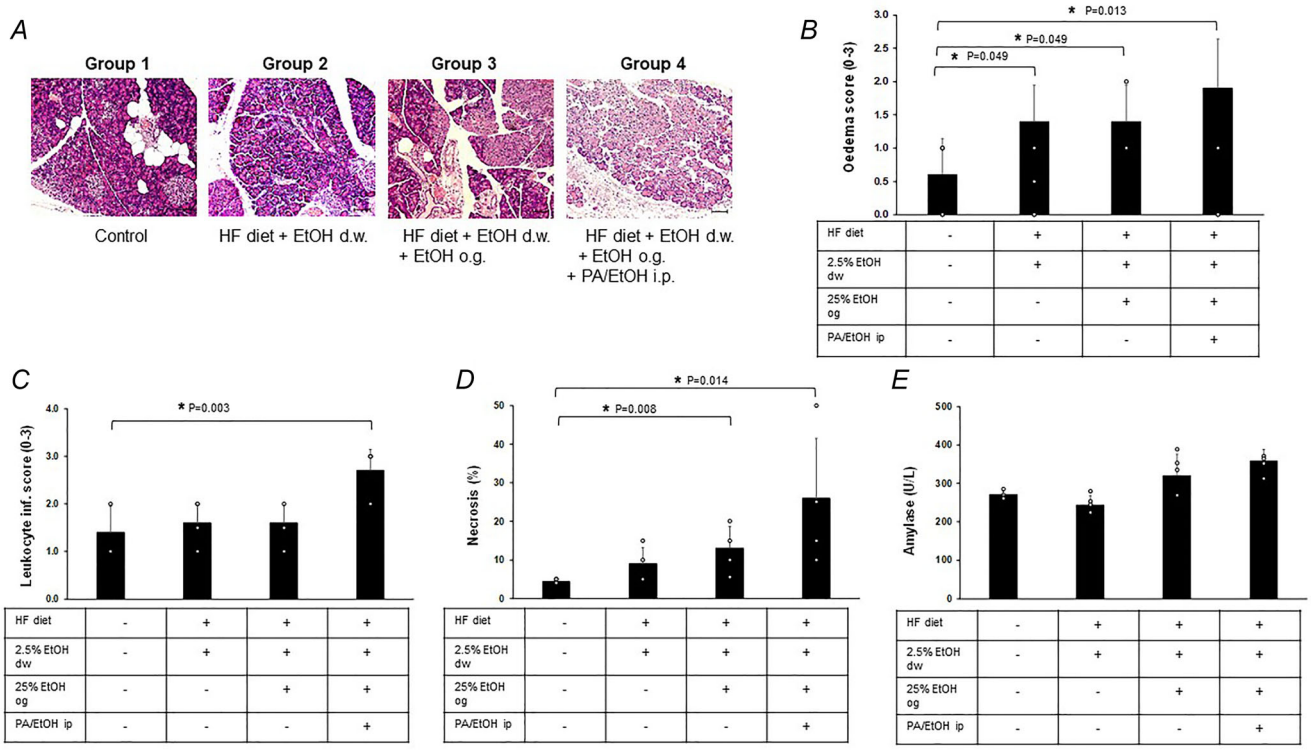

Figure 7. Effect of different ethanol treatments on the laboratory and histological parameters of guinea-pig pancreas
A, representative histological images show pancreatic sections from the control (group 1) and EtOH-treated (groups 2–4) animals. Scale bar = 20 µm. The rate of oedema (*B*), leukocyte infiltration (*C*), necrosis (*D*) and the serum levels of amylase (*E*) were measured in the guinea-pigs after induction of pancreatitis. The control animals received the same amount of physiological saline (phys. sal). Data are presented as the mean ± SD. In the case of oedema: **P* = 0.049 (group 2), *P* = 0.049 (group 3) and *P* = 0.013 (group 4) *vs.* group 1 (control). In the case of leukocyte infiltration, **P* = 0.003 (group 4) *vs.* group 1 (control). In the case of necrosis: **P* = 0.008 (group 3) and *P* = 0.014 (group 4) *vs.* group 1 (control) (*n* = 5 per group). HF diet, high-fat diet; d.w., drinking water; o.g., oral gavage tube; I.P., intraperitoneal; EtOH, ethanol; PA, palmitoleic acid.

The two active ingredients in Orkambi are VX-809 and VX-770. VX-809 (commonly known as lumacaftor) is a small molecule that has been shown to be beneficial in patients carrying a F508del homozygous mutation, in which the improper folding of the channel leads to its premature degradation (Ren et al., 2013). VX-770 (commonly known as ivacaftor) is a CFTR potentiator that increases the open time of CFTR, in both wild-type and different gating mutations, such as G551D (Gentzsch & Mall, 2018). A combination of the two drugs (Orkambi) has been shown to improve defective CFTR trafficking and function (Rehman et al., 2015; van Goor et al., 2011; Zhang et al., 2016) and it is therefore a first-line agent in the therapy of CF. Using isolated guinea-pig pancreatic ducts, we showed that VX-809 was able to restore CFTR expression after treatment with EtOH. We have previously shown that EtOH accelerates channel membrane turnover and damages protein folding, thus causing a defect in CFTR membrane trafficking. As a result, CFTR expression drops at the apical membrane (Maleth et al., 2015). Because VX-809 promotes the formation of the proper conformation of the channel and its trafficking to the apical membrane, it can be assumed that it repairs the damage caused by EtOH and thus restores the apical expression of the channel. Interestingly, treatment with VX-770 also restored the apical expression of the channel, although this was only observed at a higher concentration (10 μM) and in case of a longer incubation time (10 h) compared to VX-809. The underlying mechanism by which VX-770 increases the apical trafficking of the channel is not known. Previous studies have shown that VX-770 treatment adversely affects CFTR traffic and has a negative impact on the effect of VX-809 in F508del mutations (Cholon et al., 2014). This also suggests that the combined use of the two drugs should therefore be considered. By contrast, our results showed that VX-770 was able to restore apical expression of the channel and that a combination of the two agents was also effective, although we did not manage to achieve a greater response compared to the

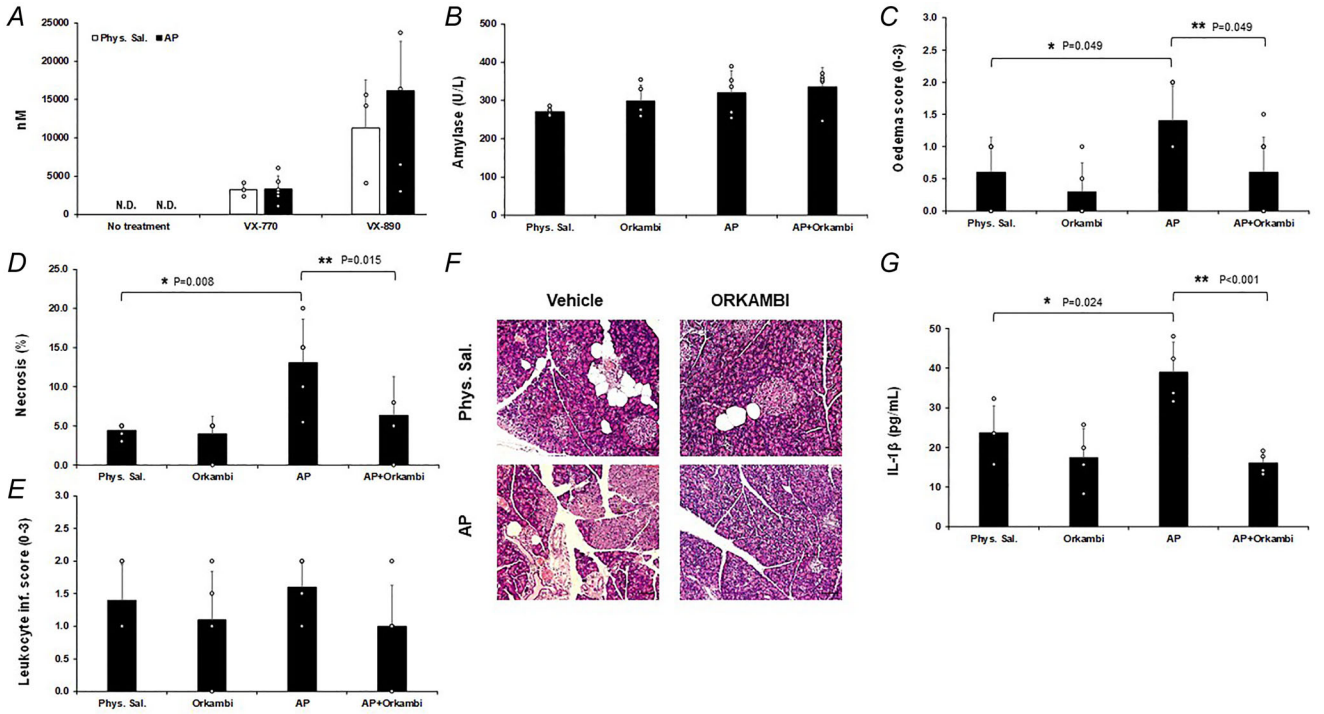

Figure 8. Effect of Orkambi on the course of alcohol-induced acute pancreatitis
A, concentrations of VX-770 and VX-809 were measured in serum samples obtained from control (phys. sal.) and EtOH-treated (AP) animals by UHPLC-MS/MS. Serum amylase activity (*B*), the rate of oedema (*C*), necrosis (*D*) and leukocyte infiltration (*E*) were measured in the guinea-pigs after induction of pancreatitis. The control animals received the same amount of phys. sal. Data are presented as the mean ± SD. In the case of oedema: $^{*}P = 0.049$ (AP) *vs*. control (Phys. Sal.). $^{**}P = 0.049$ (AP + Orkambi) *vs*. AP. In the case of necrosis: $^{*}P = 0.008$ *vs*. control (Phys. Sal.). $^{**}P = 0.015$ (AP + Orkambi) *vs*. AP. *F*, representative histological images show pancreatic sections from the control (phys. sal.) and EtOH-treated (AP) animals with or without Orkambi treatment. Scale bar = 20 μm. *G*, serum levels of interleukin-1β (IL-1β) were measured in serum samples obtained from the control (phys. sal.) and EtOH-treated (AP) animals with or without Orkambi treatment. Data are presented as the mean ± SD. $^{*}P = 0.024$ (AP) *vs*. control (Phys. Sal.). $^{**}P < 0.001$ (AP + Orkambi) *vs*. AP. Phys sal., physiological saline; AP, acute pancreatitis ($n = 5$ per group).

effect of the two agents when they were administered separately. The mechanism of action of VX-770 has been investigated in several studies (Eckford et al., 2012; Jih & Hwang, 2013; Laselva et al., 2021) focusing on channel opening. Although these studies have provided new insights into the mechanism by which VX-770 stimulates CFTR activity, other cellular effects of this compound have not been investigated. The present study is the first to show that, in addition to increasing the activity of the channel, a high dose of VX-770 can also promote CFTR trafficking to the apical membrane. Nevertheless, additional studies are necessary to clarify the underlying mechanism. Furthermore, we also examined the effects of the combined treatment at different concentrations of EtOH. The EtOH concentrations we chose correspond to moderately severe (30 mM) or severe (50 mM) intoxication in chronic alcoholics, whereas 100 mM EtOH can cause unconsciousness or even death. Our results showed that a combination of the two agents was able to restore channel expression after 30 and 50 mM EtOH treatment, although it was ineffective at 100 mM EtOH, suggesting that EtOH at this concentration already causes irreversible damage.

In the next step, we examined whether Orkambi treatment could prevent the effect of EtOH on CFTR channel activation. In our previous studies, we have shown that the basic CFTR current increases in a dose-dependent manner under the influence of EtOH, whereas the forskolin-stimulated current is significantly inhibited (Judak et al., 2014). A concentration of 30 mM EtOH was used for functional measurements because EtOH damages the channel at higher concentrations either strongly or irreversibly. Consistent with our

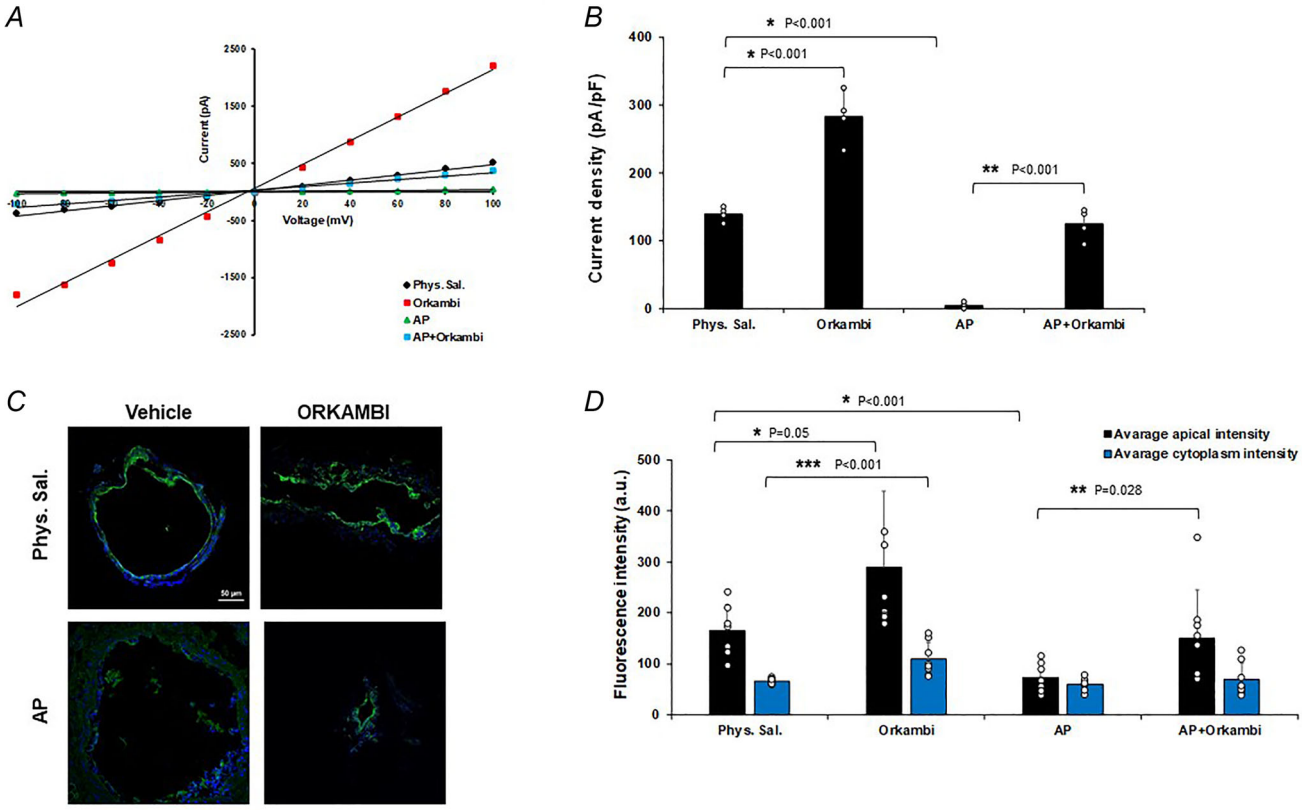

Figure 9. Activity and expression of CFTR in acute pancreatitis
A, current–voltage (*I–V*) relationships were obtained by measuring forskolin-stimulated CFTR currents over a 4 ms period starting 495 ms into the voltage pulse. *B*, summary of current densities (measured at +60 mV) in forskolin-stimulated cells. The current densities have been normalized to the cell input capacitance (pA/pF). Data are presented as the mean ± SD. $^{*}P < 0.001$ (Orkambi) and $P < 0.001$ (AP) *vs*. Phys. Sal. (control). $^{**}P < 0.001$ (AP + Orkambi) *vs*. AP (n = 4–6 per group). *C*, representative immunohistochemical staining shows the presence of CFTR in pancreatic samples from the control (phys. sal) and EtOH-treated (AP) animals with or without Orkambi treatment. Scale bar = 50 µm. *D*, summary bar chart shows the effect of AP with or without Orkambi treatment on the expression of CFTR. Mean fluorescence intensity in the ductal cells was normalized to the ductal area and expressed in arbitrary units. Data represent the mean ± SD for 5–8 specimens/five animals each group. $^{*}P = 0.05$ (Orkambi) and $P < 0.001$ (AP) *vs*. apical staining intensity of Phys. Sal. (control). $^{**}P = 0.028$ *vs*. apical staining intensity of AP. $^{***}P < 0.001$ *vs*. cytoplasm staining intensity of Phys. Sal (control). Phys sal., physiological saline; AP, acute pancreatitis.

previous studies, EtOH decreased the stimulated CFTR currents; however, treatment with VX-770 and VX-809 restored channel operation. We also found that VX-809 potentiates the effect of VX-770. This is consistent with pre-clinical studies showing that ivacaftor in combination with lumacaftor raises CFTR-mediated Cl^- conductance by several fold in F508del CFTR human bronchial epithelial cells (van Goor et al., 2011). Unlike VX-770, VX-809 did not cause a significant increase compared to the control but was able to restore channel activity after EtOH treatment. EtOH damages the CFTR channel through different mechanisms, offering different points of attack for VX-770 and VX-809. We have previously shown that the structure and assembly of CFTR, as well as its trafficking to the apical cell surface, are damaged under the influence of EtOH (Maleth et al., 2015). Moreover, EtOH damages the morphology and function of ductal mitochondria, leading to intracellular ATP depletion (Judak et al., 2014). VX-809 presumably protects against the harmful effects of EtOH by restoring channel conformation. Under physiological conditions, apical expression of the channel is not impaired, thus explaining why VX-809 does not enhance channel activity under normal conditions. However, it is able to correct EtOH-induced channel damage and thereby restore channel function. By contrast to VX-809, VX-770 binds directly to the channel and enhances its activity. ATP plays a central role in the activity of the channel; therefore, EtOH-induced ATP deficiency also contributes to reduced CFTR activity. Our results showed that VX-770 treatment was able to restore channel activity even in the absence of ATP, suggesting an ATP independent mechanism. Two independent workgroups have reported that, for certain CFTR mutations in which the mutated CFTR does not respond to ATP, such as the G551D mutation, VX-770 was able to restore channel activity, suggesting the existence of an ATP independent gating mechanism (Eckford et al., 2012; Jih & Hwang, 2013). This presumably explains why VX-770 was able to restore channel activity despite EtOH treatment.

Because the *in vitro* experiments showed promising results, the next step was to examine how Orkambi affects the course of alcoholic pancreatitis *in vivo*. In our experimental model, we used guinea-pigs because the guinea-pig pancreas has more structural and functional similarities to that of humans compared to other rodents in several aspects. In the case of the guinea-pig, the main pancreatic duct or duct of Wirsung is present, which branches into inter- and intralobular ducts, similarly to humans. By contrast, in the case of mice or rats, there is a large interlobular duct that branches toward the lobes (Dolensek et al., 2015; Gal et al., 2021). In addition, the guinea-pig pancreas is capable of secreting 140–150 mM HCO_3^- under stimulated conditions, whereas only 70–80 mM HCO_3^- is secreted in the case of rats and mice (Yamaguchi et al., 2017). Also, a unique and most important feature of the guinea-pig pancreas is that the ductal cells express CFTR in high amounts compared to rats or mice and therefore provide a suitable model for the investigation of CFTR modulators (O'Reilly et al., 2000). Despite structural and secretory differences, the most accepted and frequently used experimental AP models (cerulein and taurocholic acid) are induced in mice and rats. Therefore, as a first step, we developed a new model by which we were able to induce moderately severe, necrotizing pancreatitis in the guinea-pigs. Interestingly, although the histological image showed large necrotic areas, the serum amylase activity did not change compared to the control. Serum amylase activity is a good indicator for the diagnosis of pancreatitis. However, its value is not specific to pancreatitis and, in some cases, its level remains in the normal range even if massive tissue necrosis is present (Muniraj et al., 2015). One possible explanation for why the serum amylase level did not elevate in our experimental model could be that amylase clearance increased. In a previous study by Orda et al. (1976), acute pancreatitis was induced in guinea-pigs with sodium taurocholate, and serum levels of amylase were measured. Although some increase in amylase activity was detected, it was not significantly different compared to the control animals. This study examined the amylase-to-creatinine ratio as well, which was significantly increased in AP, suggesting increased amylase clearance. Although we did not examine amylase-to-creatinine ratio, it is conceivable that amylase clearance increased in our experimental setup also, which may explain why we did not obtain a difference in serum amylase level between AP and control animals. Furthermore, although leukocyte infiltration increased in our model, it was not significantly higher compared to the control. Orkambi treatment was administered in parallel with the oral gavage of EtOH on every third day, meaning that the animals received six Orkambi treatments during the experiment. We found that the serum levels of VX-809 were orders of magnitude higher than serum levels of VX-770 in both the control and AP animals. These results are consistent with data measured in humans, where Orkambi treatment resulted in very low plasma levels of Ivacaftor (0.06 μg mL^{-1}) but a relatively high plasma concentrations of Lumacaftor (4.42 μg mL^{-1}) (Schneider et al., 2016). Administration of Orkambi reduced the degree of necrosis and oedema and restored CFTR expression and activity as well as the serum levels of IL-1β. The favorable effect of Orkambi in alcoholic pancreatitis can presumably be explained by the fact that the restoration of CFTR function by the two components causes an increase in HCO_3^- secretion, which normalizes the intraluminal pH and the volume of pancreatic juice. Because ductal secretion plays an important role in maintaining the integrity of the pancreas

(Maleth et al., 2015; Venglovecz et al., 2008, 2018), it is conceivable that the restoration of the secretion enhances the defense ability of the pancreas against the damaging effects of EtOH and thereby reduces the severity of EtOH-induced pancreatitis.

In clinical practice, patients usually present with sudden onset of abdominal pain, and treatment is started after pancreatitis is diagnosed. In the case of acute recurrent pancreatitis (ARP), repeated episodes of AP occur, in which alcoholic aetiology plays a role. Recent studies have indicated that CFTR modulators, especially ivacaftor, can be beneficial in the prevention or reduction of ARP (Akshintala et al., 2019; Carrion et al., 2018; Johns & Rowe, 2019), which raises the possibility that Orkambi could be part of the therapy in alcoholic ARP patients, where CFTR functions are impaired.

In conclusion, the present study has demonstrated for the first time that the clinically available and approved CFTR modulator Orkambi can mitigate experimental alcoholic pancreatitis by restoring the expression and function of pancreatic ductal CFTR. This raises the possibility of using CFTR modulators in the therapy of pancreatitis, especially in the prevention of recurrent attacks. Therefore, the observations presented here encourage the conduction of clinical trials in which the effect of CFTR modulators is tested in either the prevention of pancreatic attack or in the improvement of pancreatic function in AP.

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Additional information

Data availability statement

All of the data ($n \leq 30$) supporting the findings of the present study are available within the paper.

Competing interests

The authors declare that they have no competing interests.

Author contributions

V.V. performed the patch clamp experiments, analysed and interpreted the data and drafted the manuscript. A.G. and E.T. conducted the immunostaining. E.T. and A.G. performed the pancreatitis induction and ELISA and analysed the data. K.M. and E.G. took part in the animal experiments. Z.G. and P.M. performed the UHPLC-MS/MS experiments. J.M. and Z.R. were involved in the data interpretation and edited the manuscript. P.H. supervised the project, took part in the data interpretation and edited the manuscript. All of the authors approved the final version of the manuscript submitted for publication.

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Keywords

CFTR, ethanol, Orkambi, pancreatitis

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History

Translational perspective

In recent years, the therapy of AP has improved greatly; however, the mortality rate is still unacceptably high, which presents a major challenge to doctors. Therefore, there is an urgent need for effective therapies. One of the main cell types of the exocrine pancreas is the ductal epithelial cell, which secretes a HCO_3^--rich, isotonic fluid that prevents the premature activation of digestive enzymes, thereby performing an important protective function. Heavy alcohol consumption severely impairs ductal secretion in which the reduced activity and expression of the CFTR channel plays a prominent role. Decreased ductal secretion contributes to the pathogenesis of alcoholic pancreatitis; therefore, restoration of this function may be a promising therapeutic target. Orkambi is a CFTR modulator drug that is able to improve CFTR function in certain protein defects and therefore is used in the therapy of CF patients with great efficiency. In the present study, we showed for the first time that Orkambi treatment restored the expression and activity of the CFTR channel after ethanol treatment and decreased the severity of alcohol-induced pancreatitis. Taken together, these results indicate that CFTR modulators present a novel option in the pharmacotherapy of alcohol-induced pancreatitis by enhancing pancreatic functions or preventing recurrence.