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Rescue of chloride and bicarbonate transport by elexacaftor-ivacaftor-tezacaftor in organoid-derived CF intestinal and cholangiocyte monolayers

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ABSTRACT

Background: In cystic fibrosis (CF), loss of CF transmembrane conductance regulator (CFTR)-dependent bicarbonate secretion precipitates the accumulation of viscous mucus in the lumen of respiratory and gastrointestinal epithelial tissues. We investigated whether the combination of elexacaftor (ELX), ivacaftor (IVA) and tezacaftor (TEZ), apart from its well-documented effect on chloride transport, also restores Phe508del-CFTR-mediated bicarbonate transport.

Methods: Epithelial monolayers were cultured from intestinal and biliary (cholangiocyte) organoids of homozygous Phe508del-CFTR patients and controls. Transcriptome sequencing was performed, and bicarbonate and chloride transport were assessed in the presence or absence of ELX/IVA/TEZ, using the intestinal current measurement technique.

Results: ELX/IVA/TEZ markedly enhanced bicarbonate and chloride transport across intestinal epithelium. In biliary epithelium, it failed to enhance CFTR-mediated bicarbonate transport but effectively rescued CFTR-mediated chloride transport, known to be requisite for bicarbonate secretion through the chloride-bicarbonate exchanger AE2 (*SLC4A2*), which was highly expressed by cholangiocytes. Biliary but not intestinal epithelial cells expressed an alternative anion channel, anoctamin-1/TMEM16A (*ANO1*), and secreted bicarbonate and chloride upon purinergic receptor stimulation.

Conclusions: ELX/IVA/TEZ has the potential to restore both chloride and bicarbonate secretion across CF intestinal and biliary epithelia and may counter luminal hyper-acidification in these tissues.

1. Introduction

Accumulation of viscous mucus in the respiratory, intestinal and biliary tract is a hallmark of cystic fibrosis (CF), and is the primary cause of defective mucociliary clearance in the airways and of luminal obstruction in gastrointestinal epithelia [1–4]. It was shown that proper unfolding of mucins, the polymeric glycoproteins that form the main constituent of mucus in these tissues, requires concurrent bicarbonate secretion [4,5]. Bicarbonate is transported by the CF-gene encoded cystic fibrosis transmembrane conductance regulator (CFTR) channel and by chloride-bicarbonate exchangers that are functionally coupled to CFTR [6]. In the absence of bicarbonate secretion, the mucins released by the epithelium remain densely packed and attached to the epithelial surface.

Despite the evident importance of the loss of CFTR-dependent bicarbonate transport in the pathophysiology of CF, and in contrast to the plethora of studies investigating the chloride transport defect, few studies have specifically addressed the rescue of bicarbonate transport [7]. Indeed, most functional CFTR assays currently used for diagnostic and surveillance purposes, e.g. measurement of sweat chloride, forskolin-induced swelling, intestinal current measurement (ICM) and nasal potential difference, solely

Abbreviations: CaCC, Ca^{2+}-dependent chloride channels; CFTR, cystic fibrosis transmembrane conductance regulator; ELX, elexacaftor; ICM, intestinal current measurement; IVA, ivacaftor; LUM, lumacaftor; TEZ, tezacaftor.

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measure chloride or do not discriminate between chloride and bicarbonate transport. This distinction may be of importance because molecular modeling of CFTR has indicated that access of bicarbonate and chloride to the channel pore may proceed through separate routes in the inner vestibule [8]. Consequently, it is conceivable that conformational changes in this region affect bicarbonate and chloride transport differently. Congruently, it has been shown that modulator drugs, i.e. small-molecule compounds that aim to restore mutant CFTR function by changing protein folding and/or conformation, may have different effects on chloride vs. bicarbonate transport [9].

CFTR is highly expressed in the intestinal epithelium, and in the liver it is expressed exclusively in the biliary tree [10–12]. Both these epithelial tissues secrete bicarbonate as well as chloride, stimulated by hormones that trigger cyclic AMP-dependent protein kinase-mediated phosphorylation of CFTR [10,13]. In both tissues, hyper-acidification of the luminal surface, resulting from loss of CFTR-dependent bicarbonate secretion, is thought to play a key role in the CF-typical accumulation of viscous mucus, luminal obstruction and inflammation [1–4]. Intestinal disease affects most CF patients from an early age, whereas, in contrast, cholangiopathies develop more gradually, and overt liver disease is a late and more sporadic manifestation of CF [3,11]. This may explain why few studies have addressed the effect of CFTR modulators on biliary function [12,14]. However, in view of the changing demographics of CF, effective management of liver disease is becoming increasingly important and such studies seem warranted.

In the present study we asked to what extent a combination of 3 CFTR modulators, elexacaftor (ELX), ivacaftor (IVA) and tezacaftor (TEZ), which has been shown to restore anion channel function and to improve clinical outcome in CF patients carrying the Phe508del allele, restores bicarbonate and chloride transport across intestinal and biliary CF epithelia [15,16]. For this purpose, we cultured intestinal and cholangiocyte organoids from tissue of homozygous Phe508del-CFTR patients, and assessed the effect of this drug combination on chloride and bicarbonate transport across epithelial monolayers, using a modified ICM protocol.

2. Materials and methods

2.1. Organoid culture

Organoids, generated from ileal biopsies of a CF patient (homozygous Phe508del-CFTR) and non-CF control, were maintained in medium containing the growth factors Wnt3a, Noggin, R-Spondin 1 and EGF in Matrigel matrix (Corning), according to established protocols [17]. Intrahepatic cholangiocyte organoids (ICO) were cultured from CF liver explant tissue (homozygous Phe508del-CFTR) and tissue of a deceased non-CF donor [18]. ICO were initiated and cultured in medium containing the growth factors R-Spondin 1, EGF and DKK1 in basement membrane extract matrix (BME; Cultrex), according to protocols described elsewhere [18]. To stimulate growth of CF organoids, ICO medium was supplemented with forskolin (2 μmol/L; Sigma-Aldrich). Tissue donors or their next of kin consented to tissue collection and the study was approved by the institutional review board of the Erasmus MC (MEC-2014–060).

For culture of epithelial monolayers, organoid-derived cells were seeded on a permeable substrate as described in *Supplementary material*.

2.2. Transcriptome sequencing and analysis

RNA was extracted from organoid-derived epithelial monolayers using the miRNeasy kit (Qiagen), and further sample preparation, library construction and sequencing was performed by BGI (Copenhagen, Denmark). Sequencing resulted in 22–28 million, paired-end reads. Data files were uploaded to the Galaxy public server (usegalaxy.org), quality checked (FASTQC), adapter trimmed (Cutadapt v1.16.5) and subsequently mapped against the human reference genome GRCh38 using HISAT2 (v2.1.0). Mapped reads were converted to counts (FeatureCounts v2.0.1), applying the hg38 genome annotation file, and RPKM normalized. Datasets are available through NCBI-GEO repository GSE182347.

2.3. Electrophysiological assessment of epithelial anion transport

CF and non-CF monolayers were incubated with ELX (VX-445, 3 μmol/L; MedChem), IVA (VX-770; 0.3 μmol/L; SelleckChem) and TEZ (VX-661, 3 μmol/L; SelleckChem), or vehicle (DMSO, 0.2%) for 20 h. Subsequently, filters were mounted in P2302T/P2300-type Ussing chambers (Physiologic Instruments, San Diego). Monolayers were bathed in either of 3 solutions. To assess combined chloride and bicarbonate transport, Meyler solution (mmol/L: 128 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1.0 $MgCl_2$, 20 $NaHCO_3$, 0.4 NaH_2PO_4, 0.3 Na_2HPO_4, 10 HEPES, 10 glucose) was used. For assessing chloride transport, a similarly formulated solution was used, except that $NaHCO_3$ was replaced by Na-isethionate. The pH of this solution was set at 7.35 by NaOH titration. The only CFTR-permeating anion in this solution is chloride. For assessing bicarbonate transport, NaCl was replaced by Na-isethionate, KCl by KNO_3, and $CaCl_2$ and $MgCl_2$ by acetic acid salts of these ions. The only physiologically relevant CFTR substrate in this solution is bicarbonate. The appropriate CFTR modulator combinations were added and solutions were maintained at 37 °C, gassed with 95% O_2, 5% CO_2 or O_2 only (in case a bicarbonate-free solution was used). The transepithelial potential difference was clamped at 0 mV with a VVC-MC8 module (Physiologic Instruments), and the resulting short-circuit current (Isc) was recorded using a PowerLab 8/35 recording unit and associated software (LabChart 8; AD Instruments). CFTR-mediated Isc responses in monolayers were stimulated by forskolin (10 µmol/L). UTP (50 µmol/L; Sigma-Aldrich) was added to the luminal bath to stimulate Ca^{2+}-dependent chloride channels (CaCC). UTP responses were assessed in the presence of the CFTR blocker CFTRinh172 (20 µmol/L; SelleckChem). T16Ainh-A01 (50 µmol/L; Tocris) was used to block CaCC activity.

2.4. Statistical analysis

Data are represented as means ± standard error. Differences between means were statistically analyzed by ANOVA, using the Sidak correction to control for multiple comparisons, except for the effect of T16Ainh-A01 which was statistically evaluated using Student's *t*-test (Prism 9; Graphpad software).

3. Results

3.1. Expression of ion transporters in biliary vs. intestinal epithelial monolayers

Gene expression was analyzed by transcriptome sequencing of representative CF and non-CF monolayer cultures of cholangiocyte and intestinal organoids (Fig. 1). Congruent with functional assays (see below), *CFTR* was robustly expressed in both intestinal and biliary epithelial monolayers, whereas only the latter contained a significant transcript numbers of the CaCC anoctamin-1/TMEM16A (*ANO1*). Both intestinal and biliary cells expressed the purinergic receptors $P2Y_1$ (*P2RY1*) and $P2Y_2$ (*P2RY2*). AE2 (*SLC4A2*), which is situated at the basolateral pole in intestinal cells, but mediates apical bicarbonate efflux in cholangiocytes, was ubiquitously expressed in both intestinal and biliary monolayers. Cholangiocytes also expressed the chloride-bicarbonate exchanger AE3

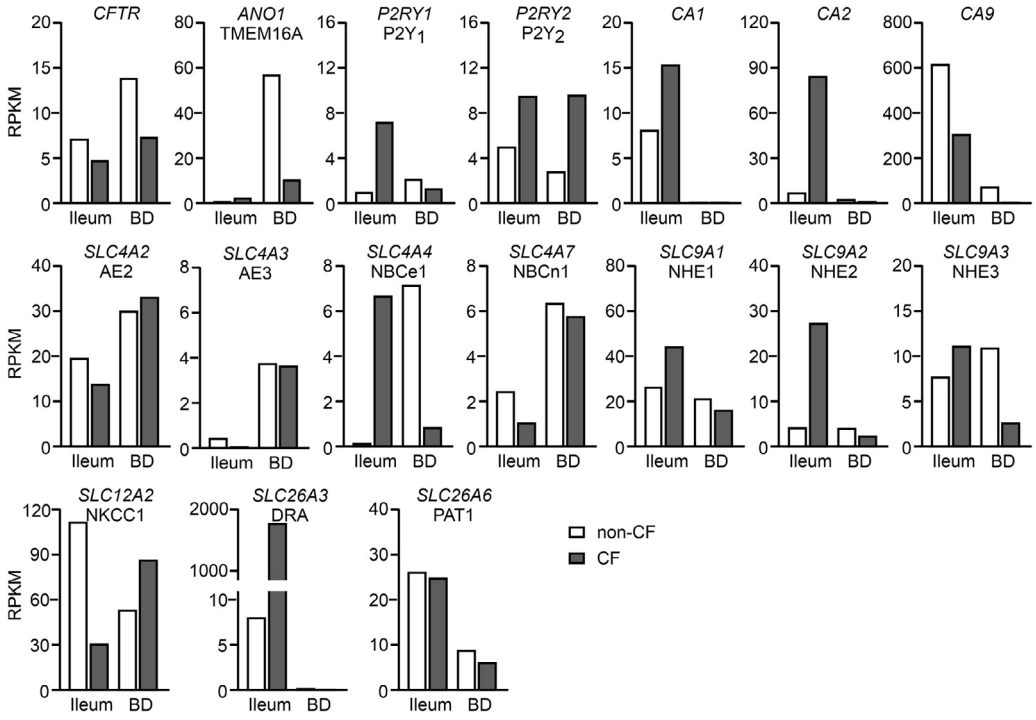

Fig. 1. Expression of genes involved in transepithelial anion transport in epithelial monolayers of organoids cultured from ileum or bile duct (BD) of CF and non-CF donors ($n = 1$ per group). Transcript levels are visualized using normalized read counts (RPKM).

(*SLC4A3*). In contrast to AE2, the chloride-bicarbonate exchangers DRA (*SLC26A3*) and PAT1 (*SLC26A6*) are located in the apical membrane of enterocytes. Intriguingly, exceptionally high levels of *SLC26A3* transcript were detected in CF monolayers. Biliary monolayers of both genotypes expressed *SLC26A6*, but contained negligible amounts of *SLC26A3* transcript. The sodium-potassium-chloride cotransporter (NKCC1; *SLC12A2*) and the sodium bicarbonate cotransporter 1 (NBCe1; *SLC4A4*) mediate cellular uptake of chloride and bicarbonate, respectively, across the basolateral membrane. In addition, both intestinal and biliary monolayers contain transcripts coding for another bicarbonate transporter, NBCn1 (*SLC4A7*). The major carbonic anhydrase in both cell types is *CA9*, but intestinal cells also contain low amounts of *CA1* and *CA2* transcripts. Members of the SLC9 family are sodium-proton exchangers that serve to counter acid loading.

3.2. ELX/IVA/TEZ partially restores Phe508del-CFTR-mediated chloride and bicarbonate transport in intestinal epithelial monolayers

The ICM technique was adapted to assess CFTR-dependent anion secretion across epithelial monolayers cultured from intestinal organoids [19]. In non-CF monolayers, forskolin elicited a robust Isc response that was blocked by CFTRinh172, indicating that the response is mediated by CFTR (Fig. 2A). Forskolin also elicited an Isc response when the assay was performed in media containing either only chloride or only bicarbonate as the CFTR-permeating anion, consistent with the notion that CFTR mediates both bicarbonate and chloride transport. However, the forskolin-mediated Isc response in bicarbonate medium amounted to only ca. one third of the response in chloride containing medium. In CF monolayers (homozygous Phe508del), the forskolin-mediated Isc responses were small, indicating low levels of residual CFTR activity (Fig. 2B, DMSO-treated). Treatment with the combination of ELX, IVA and TEZ (ELX/IVA/TEZ) significantly increased the forskolin-dependent Isc response of the CF monolayers, which reached levels amounting to ca. 50% of the response in organoids expressing wild type CFTR (Fig. 2B, 2C). ELX/IVA/TEZ enhanced both CFTR-mediated chloride and bicarbonate transport. The ratio of the Isc responses in chloride and bicarbonate medium in ELX/IVA/TEZ-treated CF monolayers (3.3 ± 0.3, $n = 6$) was similar as in non-CF monolayers (3.6 ± 0.2, $n = 6$; Fig. 2D). In accordance with low *ANO1* expression (Fig. 1), UTP did not elicit an appreciable Isc response in intestinal monolayers (not shown).

3.3. ELX/IVA/TEZ partially restores Phe508del-CFTR-mediated chloride transport in biliary epithelial monolayers

In non-CF biliary epithelial monolayers, forskolin elicited a highly transient Isc response that was followed by a substantially lower but more sustained response, amounting to 30–50% of the preceding peak Isc. Additional experiments showed that removal of chloride from the luminal bathing solution, led to a more sustained forskolin-dependent Isc response (Fig. 3A, middle panel). This suggests that in the presence of luminal chloride, the electrochemical driving force for anion extrusion is rapidly dissipated, putatively because of a comparatively low activity of chloride importers located in the basolateral plasma membrane. In non-CF biliary monolayers, other than in intestinal monolayers, the Isc response in bicarbonate medium exceeded the response in chloride medium. In CF monolayers, the forskolin-dependent Isc was considerably lower than in the non-CF specimens, but some CFTRinh172-sensitive Isc response was apparent (Fig. 3B, DMSO-treated). ELX/IVA/TEZ markedly enhanced the forskolin-dependent Isc in CF monolayers when assayed in chloride-containing medium, whereas no statistically significant effect was observed when the assay solution contained only bicarbonate as the CFTR substrate (Fig. 3B, 3C).

UTP, through activation of purinergic receptors, stimulates biliary secretion through CaCCs, independent from CFTR [20]. Indeed, in biliary monolayers, in contrast to intestinal cells, luminal

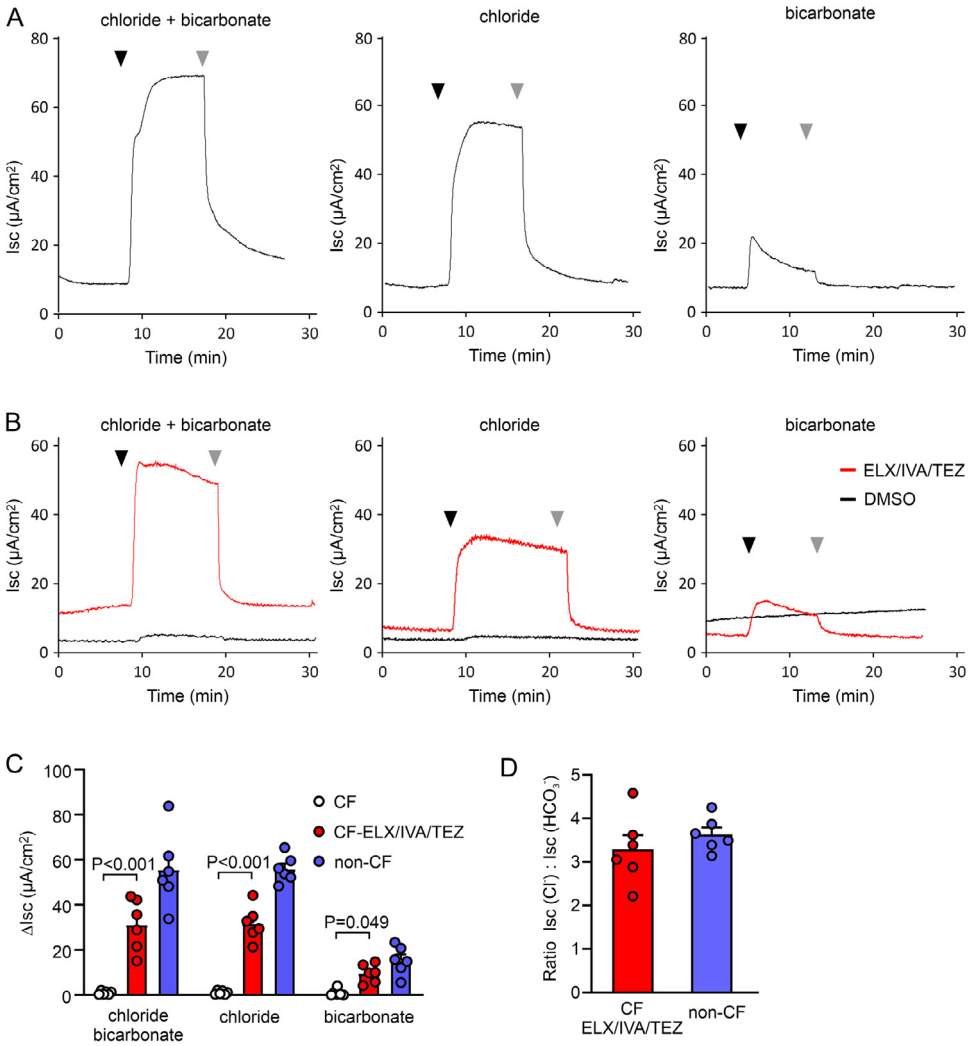

Fig. 2. CFTR-mediated Isc responses of CF and non-CF intestinal epithelial monolayers. Monolayers were bathed in medium containing both chloride and bicarbonate, only chloride, or only bicarbonate, as indicated. CF monolayers were assayed both in the presence or absence of ELX/IVA/TEZ. Forskolin was added (black arrowheads) to stimulate CFTR activity. Shaded arrowheads denote addition of CFTRinh172. A: Representative examples of non-CF monolayers. B: Representative examples of CF monolayers. C: Aggregate data showing peak forskolin-dependent Isc responses. D: Ratio of the forskolin-dependent Isc responses in chloride and bicarbonate medium. For both genotypes, organoids were derived from tissue of a single donor. Each data point represents one technical replicate ($n = 6$ for each group).

UTP elicited a substantial Isc response, which was blocked by the anoctamin-1 inhibitor T16Ainh-A01 (Fig. 3D). UTP-dependent chloride and bicarbonate secretory responses were similar in non-CF and CF monolayers (not treated with ELX/IVA/TEZ; Fig. 3E, 3F).

Because ELX/IVA/TEZ enhanced the forskolin-dependent Isc in CF monolayers only when assayed in chloride-containing medium, the ratio of the Isc responses in chloride and bicarbonate medium in ELX/IVA/TEZ-treated CF monolayers was substantially higher than in non-CF monolayers (Fig. 3G). In non-CF monolayers, the ratio of the Isc responses in chloride and bicarbonate medium after UTP stimulation was significantly higher than for the forskolin-dependent responses, and more similar to the ratio observed in CF monolayers (Fig. 3G).

4. Discussion

In this study we show that ELX/IVA/TEZ partially restored Phe508del-CFTR function in primary cultures of CF intestinal and biliary epithelium. In intestinal epithelium, ELX/IVA/TEZ significantly increased both Phe508del-CFTR-mediated chloride and bicarbonate transport. In biliary epithelium, ELX/IVA/TEZ enhanced Phe508del-CFTR-mediated chloride transport, but did not significantly improve bicarbonate transport. In contrast to intestinal cells, cholangiocytes displayed CFTR-independent, CaCC-mediated chloride and bicarbonate transport.

ELX and TEZ are small-molecule compounds that improve (co-translational) folding of the Phe508del-CFTR molecule. They bind to different regions of the nascent protein, and, when combined, increase the level of mature Phe508del-CFTR more than monotherapy with either compound [16]. ELX also acutely enhances Phe508del-CFTR-mediated chloride transport, indicating it also improves channel gating, which is further improved by IVA [21]. Administered in combination, these drugs were shown to improve CFTR-mediated chloride and fluid transport, and lung function in patients, even in those carrying only a single Phe508del allele [15,16]. However, it has not been ascertained whether this drug combination also enhances CFTR-dependent bicarbonate transport, required to counter CF-typical hyper-acidification of luminal surfaces and for detachment of mucus from the epithelial surface [4,5]. To answer this question, we assessed chloride and bicarbonate transport across epithelial monolayers derived from organoid cultures of CF (homozygous Phe508del) intestinal and biliary tissue. Other than in spheroids cultured in an extracellular matrix, both the basolateral (serosal) and the apical (luminal) com-

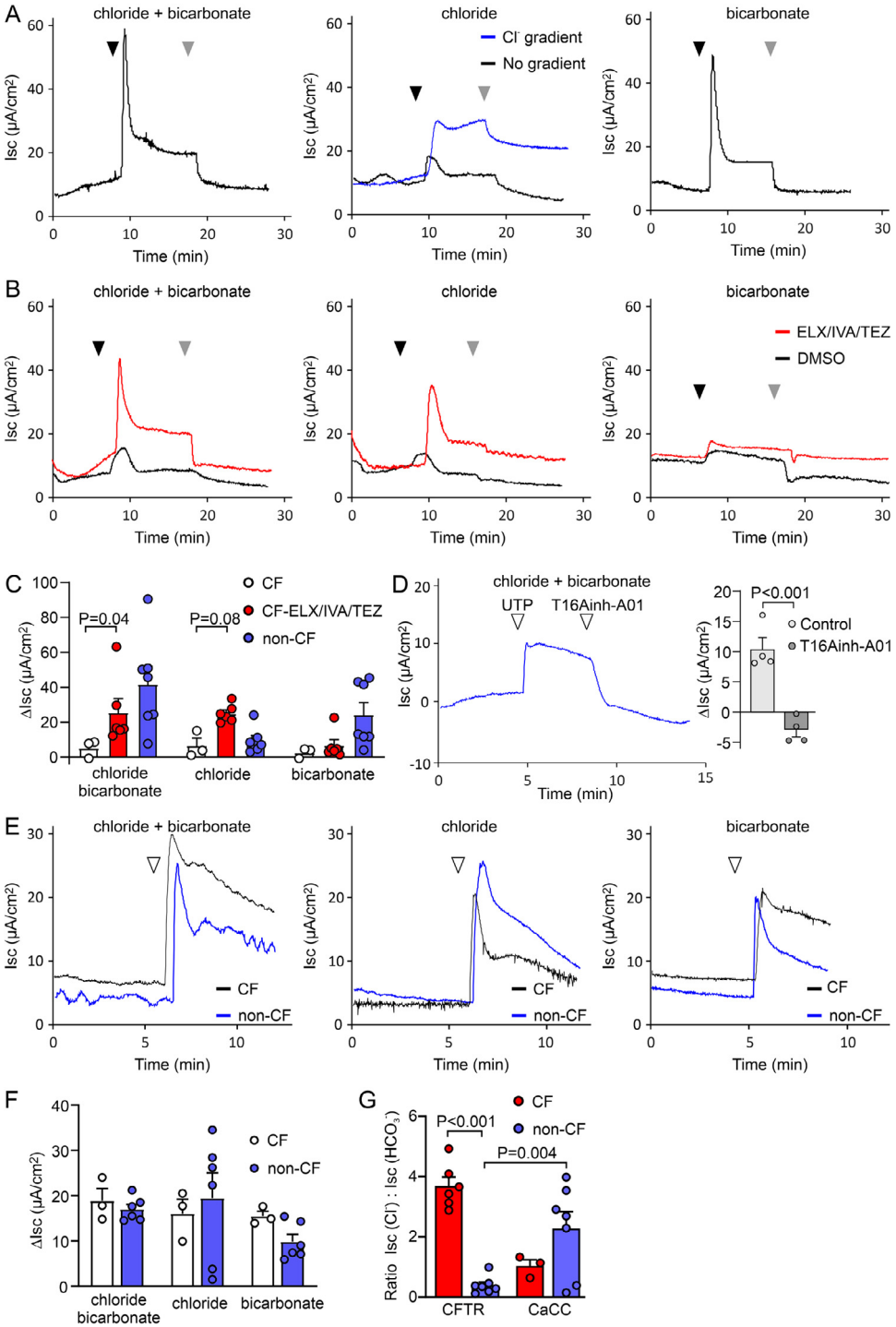

Fig. 3. CFTR- and UTP-mediated Isc responses of CF and non-CF biliary epithelial monolayers. Monolayers were bathed in medium containing both chloride and bicarbonate, only chloride, or only bicarbonate, as indicated. Non-CF monolayers were also assayed in the absence of luminal chloride. CF monolayers were assayed both in the presence or absence of ELX/IVA/TEZ. Forskolin was added (black arrowheads) to stimulate CFTR activity. Shaded arrowheads denote addition of CFTRinh172. A: Non-CF monolayers. B: CF monolayers C: Aggregate data of peak forskolin-dependent Isc responses. D: Representative experiment showing the effect of UTP and subsequent addition of T16Ainh-A01 on the Isc response of non-CF monolayers. The bar graph depicts UTP-dependent Isc responses in the presence or absence of T16Ainh-A01. E: UTP (arrowheads) was added to stimulate CaCC-mediated anion secretion across CF and non-CF monolayers. F. Aggregate data of peak UTP-dependent Isc responses. G: Ratio of the CFTR-mediated, forskolin- and CaCC-mediated, UTP-dependent Isc responses in chloride and bicarbonate medium. For CF monolayers, forskolin-dependent responses were assessed in the presence of ELX/IVA/TEZ. For both genotypes, organoids were derived from tissue of a single donor. Each data point represents one technical replicate (n = 3–7 per group).

partment of such monolayer cultures are readily accessible, enabling ion substitution experiments to assess bicarbonate transport separately from chloride transport.

In intestinal monolayers, we found that ELX/IVA/TEZ partially restored both Phe508del-CFTR-mediated chloride and bicarbonate transport, whereas CFTR-mediated Isc responses were virtually absent in untreated CF monolayers. After treatment with ELX/IVA/TEZ, both the chloride- and bicarbonate-mediated Isc response dramatically increased, reaching ca. 50% of the response observed in non-CF monolayers. This resulted in a similar chlo-

ride over bicarbonate transport ratio as observed for wild type CFTR (Fig. 2D). This ratio is comparable with values reported in studies on intestinal and respiratory epithelium, in which similar electrophysiological assays were performed, using ion substitution to distinguish CFTR-mediated chloride and bicarbonate transport [22,23]. It also closely approximates previously reported estimates for the chloride over bicarbonate permeability ratio of CFTR [24–26]. Consequently, it appears that binding of the modulator compounds changes the conformation of Phe508del-CFTR such that its ion selectivity closely mimics that of the wild type channel. Previously, it was shown that the folding correctors lumacaftor (LUM; VX-809) and TEZ (in the absence of ELX) decrease the chloride over bicarbonate permeability of Phe508del-CFTR expressed in FRT cells [9]. However, in contrast to the present study, this previous study assayed Phe508del-CFTR-mediated bicarbonate and chloride transport in the absence of IVA and ELX. Both these compounds improve the gating of the mutant channel, and it is conceivable that their co-application corrects putative effects of LUM or TEZ monotherapy on the relative bicarbonate and chloride conductance of the channel pore [21]. In fact, in our hands, LUM alone did not significantly enhance the forskolin-dependent Isc in CF monolayers, indicating that it cannot overcome the Phe508del-CFTR-typical gating defect in these patient-derived cells, possibly because they express low levels of CFTR in comparison to transfected FRT cells; LUM needs to be combined with a compound that restores CFTR channel gating, like IVA, to achieve an appreciable response.

ELX/IVA/TEZ also significantly enhanced CFTR-mediated anion secretion across biliary monolayers. It had a most pronounced effect on chloride transport, whereas it did not significantly enhance CFTR-mediated bicarbonate transport. However, cholangiocytes secrete bicarbonate mainly through an apically located electroneutral chloride-bicarbonate exchange mechanism (AE2; *SLC4A2*). According to this model, CFTR is required for extrusion of chloride entering the cells via the exchanger, enabling continued bicarbonate secretion via AE2 [27]. This model implies that restoration of Phe508del-CFTR-mediated chloride secretion suffices to restore biliary bicarbonate secretion.

Consistent with expression of purinergic receptors and a CaCC, we demonstrated that CF monolayers secrete chloride as well as bicarbonate after stimulation by UTP. Because the response was blocked by T16Ainh-A01, it is most plausibly mediated by anoctamin-1. UTP stimulated secretion in the presence of CFTRinh172 and absence of ELX/IVA/TEZ, suggesting that this pathway offers an alternative route for bicarbonate and chloride secretion that may compensate for loss of CFTR. However, previous work suggests that purinergic receptors on cholangiocytes are activated through an autocrine mechanism, and that ATP release depends on CFTR, although the mechanism of CFTR-dependent ATP secretion was not resolved [28]. Therefore, CaCC-mediated anion secretion in vivo may ultimately depend on correction of the Phe508del-CFTR defect.

Our data on non-CF biliary monolayers indicate that, apart from facilitating AE2 operation, CFTR also directly mediates bicarbonate efflux, at a rate that, under the presently used conditions, can exceed chloride transport. Unexpectedly, CFTR-mediated bicarbonate transport was almost absent from CF monolayers, both before and after ELX/IVA/TEZ treatment. The cause of this disparity is speculative. Firstly, we cannot exclude the remote possibility that ELX/IVA/TEZ specifically promotes the permeation of chloride through the channel pore in biliary monolayers, but does not restore the permeation of bicarbonate. If so, it is unlikely to be a direct effect of ELX/IVA/TEZ on CFTR folding or anion conductance as the apparent shift in the chloride over bicarbonate transport ratio was not observed in similarly treated intestinal monolayers. However, it remains possible that in non-CF biliary monolayers, but not in the CF monolayers, intracellular chloride concentrations reach a sufficiently low level to activate WNK1 and NBCe1, chloride-sensing proteins capable of promoting CFTR-mediated bicarbonate transport [29]. Furthermore, transcriptome analysis showed that, in biliary CF monolayers, expression of NBCe1 and the principle carbonic anhydrase CA9 was substantially lower than in non-CF monolayers. However, differential gene expression cannot fully explain the disparity in transport properties between genotypes, because the low expression of these genes in the CF cells did not impose a similar limit on CaCC-mediated bicarbonate transport. Rather, this implies that the regulation of components of the bicarbonate secretory route, e.g. NBCe1, of which the surface expression is controlled by the WNK/SPAK- and IRBIT pathways, differs between the Ca^{2+} and cAMP signaling pathways, or that CFTR and the CaCC are expressed in different cell types [29,30]. Further, we cannot rule out the possibility that the marked differences in CFTR-mediated bicarbonate transport between CF and non-CF cholangiocyte organoids reflect inter-individual differences, implying that studies on additional CF donor tissue and organoids are indicated. Finally, because the conditions during in vitro cell culture can never fully recapitulate circumstances in vivo, the expression and function of ion transporters may differ between organoids/monolayers and native tissue.

5. Conclusion

Our data indicate that ELX/IVA/TEZ strongly enhances Phe508del-CFTR-mediated chloride and bicarbonate transport in (small) intestinal epithelial cells, and chloride secretion in biliary epithelial cells. Because CFTR-mediated chloride efflux from both enterocytes and cholangiocytes is thought to promote further bicarbonate secretion through coupling to electrically silent chloride-bicarbonate exchangers (which are not assessed in ICM), the actual level of bicarbonate secretion upon CFTR activation is probably substantially higher than is apparent from the Isc responses [10,13,27]. Consequently, this combination of CFTR modulators is likely to counter luminal hyper-acidification not only in the intestine, but also in the biliary tract.

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Declaration of Competing Interest

The authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2021.12.006.

CRediT authorship contribution statement

Marcel J.C. Bijvelds: Conceptualization, Investigation, Formal analysis, Visualization, Writing – original draft. **Floris J.M. Roos:** Investigation, Formal analysis, Writing – original draft. **Kelly F. Meijsen:** Investigation. **Henk P. Roest:** Data curation, Formal analysis. **Monique M.A. Verstegen:** Supervision, Writing – review &

editing. **Hettie M. Janssens:** Resources. **Luc J.W. van der Laan:** Resources, Writing – review & editing, Funding acquisition, Supervision. **Hugo R. de Jonge:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision.

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