Journal of Cystic Fibrosis 23 (2024) 1167–1172

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Original Article

Tezacaftor is a direct inhibitor of sphingolipid delta-4 desaturase enzyme (DEGS)

Dinu Zinovie Ciobanu [a], Nara Liessi [a], Valeria Tomati [b], Valeria Capurro [b], Sine Mandrup Bertozzi [a], Maria Summa [c], Rosalia Bertorelli [c], Nicoletta Loberto [d], Dorina Dobi [d], Massimo Aureli [d], Lucilla Nobbio [e], Tiziano Bandiera [f], Nicoletta Pedemonte [b], Rosaria Bassi [d], Andrea Armirotti [a,*]

[a] Analytical Chemistry Facility, Istituto Italiano di Tecnologia, Via Morego 30, 16163, Genova, Italy
[b] UOC Genetica Medica, IRCCS Istituto Giannina Gaslini, Via Gerolamo Gaslini 5, 16147, Genova, Italy
[c] Translational Pharmacology Facility, Istituto Italiano di Tecnologia, Via Morego 30, 16163, Genova, Italy
[d] Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Via F.lli Cervi 93, 20054, Segrate, Milano, Italy
[e] IRCCS Ospedale Policlinico San Martino, Largo Rosanna Benzi 10, 16132, Genova, Italy
[f] D3 PharmaChemistry, Istituto Italiano di Tecnologia, Via Morego 30, 16163, Genova, Italy

ARTICLE INFO

Keywords:
Cystic fibrosis
ETI
Tezacaftor
Dihydroceramides
Myelin

ABSTRACT

Background: We recently demonstrated that 48 h exposure of primary human bronchial epithelial (hBE) cells, obtained from both CF (F508del homozygous) and non-CF subjects, to the triple drug combination Elexacaftor/Tezacaftor/Ivacaftor (ETI) results in a CFTR genotype-independent modulation of the *de novo* synthethic pathway of sphingolipids, with an accumulation of dihydroceramides (dHCer). Since dHCer are converted into ceramides (Cer) by the action of a delta-4 sphingolipid desaturase (DEGS) enzyme, we aimed to better understand this off-target effect of ETI (i.e., not related to CFTR rescue)

Methods: hBE cells, both F508del and wild-type, were cultured to create fully differentiated bronchial epithelia. We analyzed Cer and dHCer using an LC-MS based method previously developed by our lab. DEGS expression levels in differentiated hBE cells lysates were quantified by western blot analysis.

Results: We demonstrated that 1) dHCer accumulate in hBE with time following prolonged ETI exposure, that 2) similar inhibition occurs in wild-type primary human hepatocytes and that 3) this does not result in an alteration of DEGS expression. We then proved that 4) ETI is a direct inhibitor of DEGS, that 5) Tezacaftor is the molecule responsible for this effect, that 6) the inhibition is concentration dependent. Finally, after repeated oral administration of ETI to naïve, non-CF, mice, we observed a slight accumulation of dHCer in the brain.

Conclusions: We believe that further investigations on Tezacaftor should be envisaged, particularly for the use of ETI during pregnancy, breastfeeding and in the early stages of development. DEGS dysfunction and dHCer accumulation causes impairment in the development of the nervous system, due to a derangement in myelin formation and maintenance.

1. Introduction

The triple drug combination Elexacaftor/Tezacaftor/Ivacaftor (Elexa/Teza/Iva or ETI) is a highly effective treatment, which has dramatically improved the expectancy and quality of life of many people with CF (pwCF). The drug is taken daily in the morning, in association with a high fat meal, with an additional dose of Ivacaftor later during the day. Despite being a generally well-tolerated drug, some side effects are reported, ranging from severe acute liver dysfunctions (see EMA product information file [1]) to abdominal pain, diarrhea and nausea (in up to 69% of subjects [2]) to "*brain fog*" [3]. Upon ETI treatment, a significant percentage of pwCF experiences debilitating neuropsychiatric adverse effects [4]. The attention toward this drug remains high in the scientific community and several safety studies are currently ongoing. Both EMA and FDA files on ETI (trade names: Trikafta in USA and Kaftrio in Europe) [1,5] report several interesting information, specifically for

* Corresponding author.
E-mail address: andrea.armirotti@iit.it (A. Armirotti).

https://doi.org/10.1016/j.jcf.2024.05.004
Received 28 February 2024; Received in revised form 20 April 2024; Accepted 13 May 2024
Available online 24 May 2024

Tezacaftor. This molecule has a good oral bioavailability in rats and dogs (40–50%), with maximum blood levels reached in the 2–8 h after administration. While no major sign of drug accumulation was observed in any of the tested animal models, Teza widely distributes into the body (lungs, liver and brain) and it passes through the blood-brain-barrier [6]. Quite interestingly, placental transfer of the drug, although low, is observed and it has been recently demonstrated that ETI is detectable in the blood of babies born from mothers with CF undergoing treatment [7]. This is of relevance, because women with CF are increasingly planning pregnancy and the number of babies born from mothers undergoing ETI is increasing, thanks to the undeniable efficacy of this drug. In many cases, pregnant women with CF decide not to suspend the treatment. On the other hand, the only paper reporting data on ETI safety during pregnancy, while generally reassuring, just refers to "45 ETI exposed" pregnancies [8]. Moreover, after dedicated safety studies [9], in April 2023 FDA approved the use of ETI in children from 2 to 6 years old. [6]. We have recently reported that ETI is able to alter the balance between dihydroceramides and ceramides in the human bronchial epithelium in a genotype-independent way, with cells from CF and non-CF subjects showing the same behavior [10]. In the *de novo* synthetic pathway of sphingolipids, ceramides (Cer) are produced from dihydroceramides (dHCer) by the action of sphingolipids desaturase (*DEGS*), a sphingolipid delta(4)-desaturase (DEGS) that is able to oxidize an N-acyl sphinganine at position 4 to an N-acyl sping-4-enine, i.e., a ceramide [11]. DEGS1, the most abundant enzyme, [11] only converts dHCer into Cer while DEGS2 also acts as a monooxygenase [11,12] producing 4-hydroxyceramides (phytoceramides) from dHCer. It is also assumed that DEGS has similar activity on dihydrosphingolipids of virtually all chain lengths, although there is a lack of experimental evidence mainly due to the difficulty in obtaining meaningful data in in vitro assays. In fact, dihydrosphingolipids have huge solubility issues in aqueous buffers [13], in particular the longer sphingoid bases (longer sphingoid bases are less and less soluble in water). Another important activity of DEGS1 lies in its vitamin A isomerase activity [14]: this enzyme also converts trans-retinol into cis-retinol in retinal cells and it thus plays a significant role in the mechanism of vision. DEGS severe malfunction results in accumulation of dHCer in tissues [15] and accumulation of dHCer is linked to neurodegeneration [11,16,17], with a loss of white matter and defective myelin formation or maintenance [84]. Furthermore, DEGS alterations induce increased susceptibility to epilepsy [11,16,18]. In the present work, we aimed at expanding our previous observations on the inhibitory effect of ETI on DEGS1 activity. We first characterized the time-profile of the dHCer/Cer ratio modulation by ETI and we proved that dHCer accumulation is not limited to bronchial cells, but also observed in human hepatocytes. We then found that DEGS expression is not affected by ETI and, by using an in vitro functional assay, we demonstrated that Tezacaftor is able to directly inhibit DEGS in a concentration-dependent way. Finally, we translated our findings in vivo and we showed that prolonged oral administration of ETI to mice results in a minor, but significant, accumulation of dHCer in the brain.

2. Materials and methods

Please see the Supplementary Material for a detailed discussion of all Materials and Methods

3. Results and discussion

To refine our previous observations [10], we exposed human bronchial epithelial (HBE) cells derived from three F508del homozygous CF subjects and three non-CF subjects to vehicle alone (DMSO; as control condition) and/ or ETI (3,10 and 5 μM for Elexacaftor, Tezacaftor and Ivacaftor, respectively) up to 14 days, following the experimental workflow shown in Suppl. Figure **S1**. For each of these timepoints we measured the dHCer/Cer ratio. The results are reported in Fig. 1.

Exposure to ETI produces a steady accumulation of dHCer in HBE cells and it is confirmed that exposure for 24–48 h roughly doubles the amount of dHCer, while two weeks of continuous ETI exposure induces a marked and significant accumulation of these lipids compared to DMSO alone. We then explored the possibility that ETI might induce a decrease in the levels of expression of DEGS in the HBE while acting as CFTR modulator. To this purpose, the same CF and non-CF epithelia were lysed at indicated timepoints and subjected to SDS-PAGE followed by immunoblot to evaluate both CFTR maturation and DEGS1 protein expression (Fig. 2).

First, we verified ETI efficacy in improving the maturation of mutant CFTR. As expected, in lysates of epithelia derived from F508del homozygous subjects, treated with vehicle alone, the prevalent form was the immature, core-glycosylated CFTR protein (band B). Treatment with ETI rescued mutant CFTR as shown by the appearance of the mature, fully glycosylated form of the channel (band C) (Fig. 2A, C upper panel). Indeed, the fraction of the mature protein (calculated over the total amount of protein, i.e. band C over band C + band B) displayed a 4- to 16-fold increase. On the contrary, in total cell lysates of epithelia derived from non-CF subjects, under resting condition, the mature form of CFTR (band C) was the most represented, with only a small amount of the immature form. Upon ETI treatment, the fraction of the mature protein, with respect to total protein, was not altered (Fig. 2B-D upper panel). Then, we evaluated whether ETI treatment influenced the expression of DEGS1. As shown in Fig. 2 (A, C lower panel and B, D lower panel), in both CF and non-CF cells prolonged ETI treatment does not alter the levels of expression of DEGS, and it can thus be envisaged that ETI is indeed a direct inhibitor of this enzyme. We thus investigated the inhibitory activity of ETI on DEGS, by using an in-vitro functional assay described in 1997, when DEGS activity was characterized [13]. We used human liver microsomes (commercially available) as source of DEGS and we monitored the formation of d18:1/12:0 Cer from its precursor

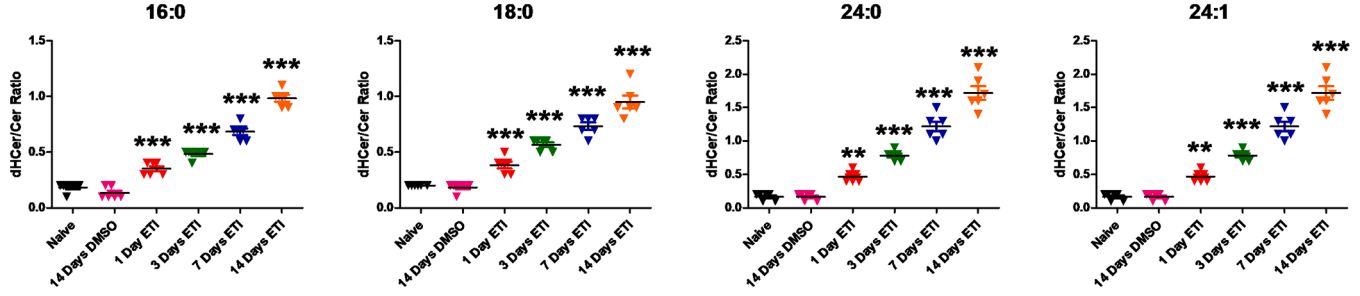

Fig. 1. dHCer/Cer ratio in HBE cells exposed to ETI for up to 14 days for four abundant sphingolipid species. Cells were obtained from 3 non-CF and 3 CF (homozygous F508del mutation) individuals. Both genotypes show the same trend for a steady accumulation of dHCer over time. *** and **= $p < 0.01$ and $p < 0.01$ respectively compared to cells exposed to DMSO for 14 days in a one-way ANOVA. ETI was administered at the same 3, 10 and 5 μM concentration for the three drugs respectively.

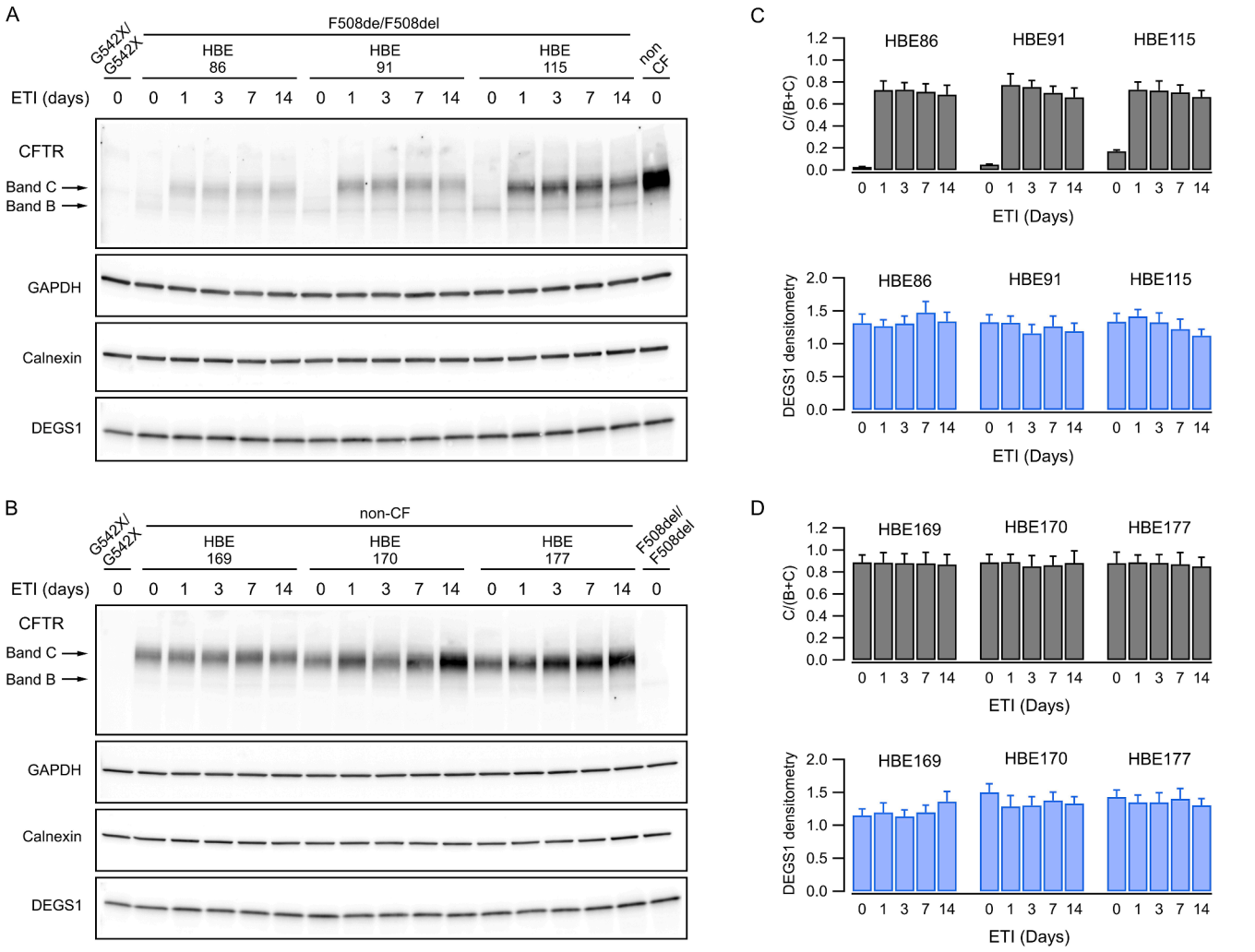

Fig. 2. Biochemical analysis of CFTR and DEGS1 protein expression pattern. **A-B** Representative western blot images showing CFTR and DEGS1 electrophoretic mobility in lysates of bronchial epithelia derived from three subjects homozygous for F508del-CFTR (in **A**, Donor ID: HBE86, HBE91, HBE115) and three non-CF individuals (in **B**, Donor ID: HBE169, HBE170, HBE177). Prior to lysis, epithelia were treated with ETI or DMSO as depicted in the experimental workflow in Suppl. Figure **S1**. Lysates of nasal epithelia derived from a CF donor homozygous for G542X variant (ID: GE220) have been included as negative control. In the last lane of panel A lysates of HBE170 epithelia (indicated as non-CF) treated with vehicle alone for 14 days has been included as control. In the last lane of panel B lysates of HBE91 epithelia (indicated as F508del/F508del) treated with vehicle alone for 14 days has been included as control. Arrows indicate the complex-glycosylated (band C) and core-glycosylated (band B) forms of the CFTR protein. **C-D** Bar graphs showing CFTR maturation (expressed as C/(C + B) band ratio; upper panels) and DEGS1 densitometry (lower panel) of the western blot experiments shown in A and B, respectively. Error bars means ± SD.

d18:0/12:0 dHCer, used as exogenous substrate, over 3 h. The reaction was initiated by the addition of NADH. Fenretinide, a known DEGS inhibitor [19,20] that reached clinical trials for CF [21,22] and cancer treatment [23] as modulator of ceramide metabolism, was used as positive control. DMSO was used as negative control. The results are reported in Fig. 3.

As clearly demonstrated by the functional assay, ETI is a direct inhibitor of DEGS, able to slow down its ability to convert dHCer into Cer with an efficacy comparable to that of Fenretinide. By using the same assay, we then investigated the effect of each of the three ETI components individually and, as reported in Fig. 4A, we identified Tezacaftor as the (sole) molecule responsible for the observed inhibition.

Indeed, the level of DEGS inhibition in the presence of Tezacaftor alone (at 10 μM) does not differ from that observed in the presence of ETI, while Elexacaftor and Ivacaftor did not show any effect on DEGS activity. We then went one step further and explored the concentration/inhibition profile of Tezacaftor. As reported in Fig. 4B, this drug inhibits DEGS in a concentration dependent manner, with an IC50 around 3 μM, a concentration that caused a decrease in the enzymatic activity around 50–60 %. Our data clearly indicate that Tezacaftor is a direct inhibitor of DEGS and that, following continuous administration of ETI, an accumulation of dHCer is clearly observable. Based on the in vitro data we collected on HBE and on liver microsomes, 3 μM Tezacaftor decreases DEGS activity to roughly 50 %. To better assess the real impact of our findings on the main players of human lipid and drug metabolism, we also tested the ability of ETI and Tezacaftor to inhibit DEGS in cultured human hepatocytes. **Figure S2** shows the dHCer/Cer observed in cultured human (non-CF) hepatocytes following 48 h exposure to ETI and Teza. This data also helps us in putting our findings in the right context from the drug safety standpoint. The dHCer accumulation triggered by ETI and Tezacaftor is significant, but overall lower than that elicited by Fenretinide, a drug that underwent clinical trial for cancer and that failed for lack of oral bioavailability, not for safety issues. Moreover, the hepatocytes did not show any significant difference in LDH release after 48 h treatment with ETI, Tezacaftor or Fenretinide compared to control cells (**Figure S3**), thus indicating that the drug did not affect the viability of hepatocytes. Indeed, the overall cell viability at the end of the experiment was not less than 85 %. Finally, we

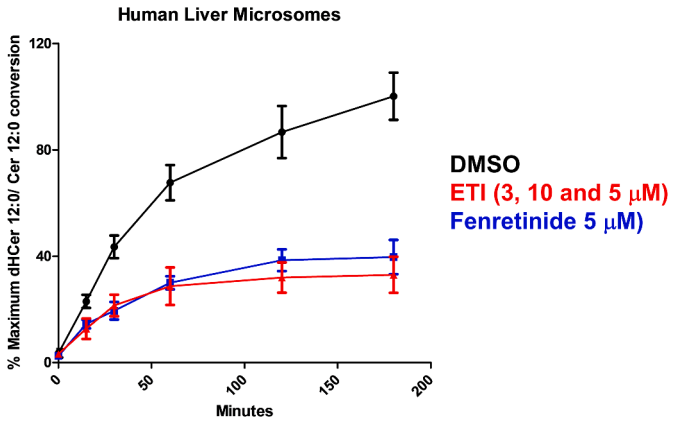

Fig. 3. Time profile of the formation of Cer 12:0 from the substrate dHCer 12:0 in human liver microsomes. DMSO was used as negative control, 5 μM Fenretinide as positive control. ETI was tested at the same concentrations used for the experiment on BE cells. DMSO, Fenretinide and ETI were incubated with the microsomes for 30 min. prior to the addition of NADH (cofactor that starts the reaction). Data refers to 5 independent replicates and are plotted as average +/- standard deviation. Incubation was done at 37 °C under gentle shaking.

investigated if the data we collected in vitro could somehow be observed in in vivo settings. To this purpose, we administered ETI orally to naïve, non-CF, male CD1 mice twice a day for 4.5 days at the same pro-kilo dose used for adult humans (3, 1.5 and 2.3 mg/Kg, assuming a body weight of 65 Kg). We then quantified the ETI compounds in the plasma of these animals at the end of the experiment. As summarized in Table S1, while Elexacaftor levels are comparable to those reported for CF adult subjects undergoing Kaftrio [24], Tezacaftor and Ivacaftor show markedly lower plasma concentrations. Given the critical role of dHCer accumulation in neurodegeneration described above, we thus measured ETI and dHCer in the brain of these animals. Elexacaftor, Tezacaftor and Ivacaftor were detected in the brain at 424±133, 8.2 ± 3.0 and 1.2 ± 0.9 ng/g brain respectively and, as shown in Fig. 5, despite the overall low Tezacaftor concentration we observed in the brain, a small but significant increase in dHCer levels, compared to vehicle-treated animals, for longer-chain species (24:0 and 24:1) was observed.

The results we obtained in mice are intriguing: if we assume for brain tissue the same density as water, the Teza levels we observed (as ng/g) would virtually translate into a concentration of around 15–20 nM. This concentration is roughly 50 times lower than that eliciting any observable DEGS inhibition in our microsomal assay. On the other hand, the experimental conditions for this assay, i.e., microsomes suspended in aqueous buffer, are less than satisfactory to determine the real inhibitory potency of Teza, which has a very low water solubility. We hypothesize that the actual amount of Teza interacting with DEGS (inside the microsomes) is definitely lower than that calculated to be present in solution, given its low solubility. This might be the reason why, on the contrary, a repeated, prolonged oral administration of Teza for five days results in a minor but observable dHCer accumulation in the brain. In-vivo, Teza's solubility is increased and it is transported to the brain thanks to its binding to plasma proteins (99 % protein binding based on DrugBank data); therefore, its levels in the brain might high enough to inhibit DEGS. In other words, for unavoidable biases, our in vitro assay might underestimate the inhibitory activity of Teza on DEGS.

4. Conclusions

We have shown here that Tezacaftor is a direct inhibitor of DEGS and that the inhibition occurs at concentrations even lower than those

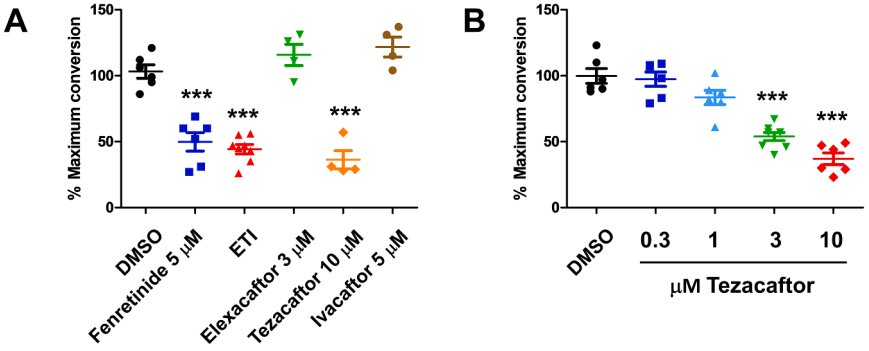

Fig. 4. A) DEGS inhibition after 3 h incubation of dHCer 12:0 substrate. DMSO was used as negative control, 5 μM Fenretinide as positive control. The ETI drugs were tested both in combination (all three drugs simultaneously) and individually, always at the same concentrations used for the experiment on HBE cells. ***= $p < 0.01$ Vs DMSO in a 1way-ANOVA +Dunnett's test. Data derives from $N = 4$ to 8 independent replicates. Fig. 4, **B)** DEGS inhibition by different concentrations of Tezacaftor after 3 h incubation of dHCer 12:0 substrate. DMSO was used as negative control. ***= $p < 0.01$ Vs DMSO in a 1way-ANOVA +Dunnett's test. Data derive from $N = 6$ to 8 independent replicates.

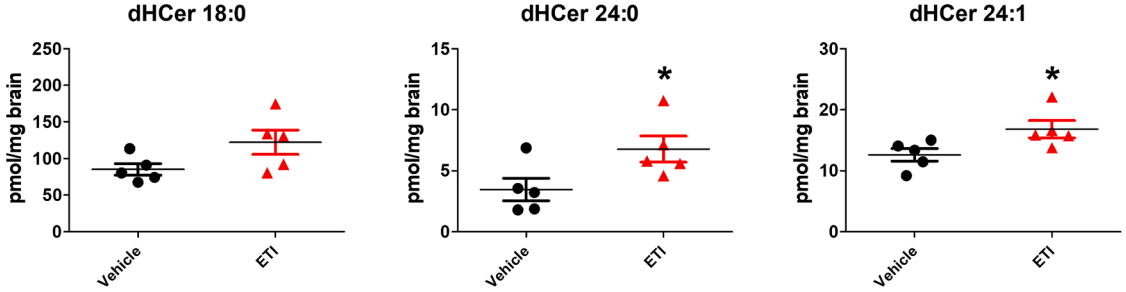

Fig. 5. dHCer levels of three abundant sphingolipid species observed in the brain of mice orally exposed to ETI for 4 days versus control animals treated with vehicle. *= $p < 0.05$ in an unpaired *t*-test Vs DMSO. Data refers to five animals per group.

reached by this compound in the plasma of adults and children undergoing ETI therapy [24]. At 3 μM Tezacaftor, DEGS activity in vitro is slowed down by around 50 %. Since ETI is assumed daily, a continuous inhibition of DEGS, with a corresponding accumulation of dHCer is thus a realistic scenario for CF individuals. Indeed, our data on prolonged exposure of cultured HBE (Fig. 1) to ETI goes in this direction, clearly showing that dHCer *do* accumulate with time. This accumulation does not immediately translate into toxicity in hepatocytes, but we here prove that prolonged oral administration of ETI to mice translates in a minor, yet significant, dHCer accumulation in the brain. Still uncharacterized is the potential inhibitory activity of Teza on DEGS2, if any. While its levels of expression are low in brain, lungs and liver [25], we cannot rule out that a potential inhibition of this enzyme might play a significant role in the intestine and skin, where DEGS2 is expressed. In this case, further studies would be needed to monitor the abundance of phytoceramides, the main products of DEGS2 activity. From the standpoint of safe use of ETI, particularly during pregnancy and in the early developmental stages, we believe that our evidences on DEGS inhibition should be further investigated. Indeed, Tezacaftor can cross both the placental and the blood-brain barriers and pass through the mother's milk [7]. It can thus be reasonably concluded that newborns from mothers undergoing ETI treatment are exposed to Tezacaftor from the very beginning of the pregnancy. This aspect triggers some potential safety concerns. DEGS activity is crucial in controlling the correct molecular composition (and structure) of myelin, a unique lipid-rich membrane structure, with sphingolipids as critical components. During myelination, distinct changes in both the nature of the sphingoid backbone and N-acyl chains incorporated into sphingolipids occur and dysregulation in enzymes involved in this complex pathway leads to dys/demyelinating phenotype [26,27]. In humans, these processes occur very early in development. The peripheral nervous system (PNS) develops between 4- and 6-weeks of gestation, with nerve fibers growing out from neuroblasts and neural crest cells in the spinal cord and dorsal root ganglia. PNS myelination begins at approximately 15 weeks of gestation [28]. Central nervous system (CNS) myelination begins as early as 12–14 weeks gestation in the spinal cord, and it continues into the third decade after birth in intracortical fibers of the cerebral cortex, but the most rapid and dramatic changes occur between mid-gestation and the end of the second postnatal year in the CNS, with myelination accounting in large part for the over tripling in brain weight during this period [29,30]. While toxicity studies on Tezacaftor in adult animals did not prompt any major alert, it is intriguing (and worrisome) that the EMA file reports that "*Juvenile toxicity studies of Teza in rats exposed during postnatal day 7 to 35 (PND 7–35) showed mortality and moribundity, even at low doses. Findings were dose related and generally more severe when dosing with Teza was initiated earlier in the postnatal period*". Such adverse effects disappear if the compound is administered *after* day 21 post birth. It is worth reporting that in rodents the first **three weeks after birth** [31] are crucial for the myelination process and the correct development of CNS and PNS. The lethal side effects observed in rats when Tezacaftor is administered in this time window might thus be consistent with DEGS inhibition, with subsequent accumulation of dHCer and impairment of myelin formation. Overall, the body of data available so far on the use of ETI in humans would exclude short/midterm safety concerns, but the story of the use of this drug during pregnancy and in infancy is too short to derive reliable conclusions, particularly related to mild alterations that might produce effects later in life. On the other hand, it is reported that patients with severe DEGS mutation [18] (virtually no enzymatic activity) showed no signs of abnormal neuronal development until the onset of the phenotype (18–24 months of age). In our data, we observed a reduction of DEGS activity to around 50 %: dHCer accumulation during ETI exposure is thus expected to be significantly lower and clinical alterations, if any, might occur significantly later in life. In this respect, further, dedicated safety studies for ETI should be carried out. If dHCer accumulation is indeed observed in the early development stages, then we believe that the use of ETI during pregnancy and breastfeeding should be at least regarded with some caution. More in general, the use of ETI in early development, if continued, would perhaps need to be associated with surveillance on specific neurodevelopmental readouts, to help the clinicians to define the best therapeutic strategy for each CF individual. The impact of our findings is likely negligible or inexistent on adults CF subjects. In their case, CNS and PNS are already developed, and the risk/benefits tradeoff allows a safe continuation of ETI therapy. In any case, further investigation on DEGS inhibition in the CF population undergoing ETI should be considered.

Credit author statement

- Dinu Ciobanu, Nara Liessi, Valeria Tomati, Valeria Capurro, Sine Mandrup Bertozzi, Maria Summa, Nicoletta Loberto, Dorina Dobi: investigation.
- Rosalia Bertorelli, Massimo Aureli, Lucilla Nobbio, Tiziano Bandiera: writing, review and editing.
- Rosaria Bassi, Nicoletta Pedemonte: formal analysis.
- Andrea Armirotti: supervision.

Declaration of competing interest

The Authors declare that no conflict of interest exists.

Acknowledgements

This study received funding from Fondazione Fibrosi Cistica, with grant FFC#1–2023 to AA. NP acknowledges Fondazione per la Ricerca sulla Fibrosi Cistica grant FFC #10/2021 (with the contribution of "Delegazione FFC Ricerca di Genova", "Delegazione FCC Ricerca Altomilanese", "Gruppo di sostegno FFC Ricerca di Campiglione Fenile", and "Delegazione FFC Ricerca di Napoli"). Work in NP lab is also supported by the Italian Ministry of Health through Cinque per mille and Ricerca Corrente and by grant GR-2018–12367126 to VT. We wish to thank Dr. Carlo Castellani for the helpful discussion.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2024.05.004.

References

[1] EMA KAFTRIO. EPAR medicine overview. 2022.
[2] P245 Kaftrio® Impact and Side-effect Survey (KISS). J Cyst Fibros 2022.
[3] Aspinall SA, et al. Evaluating the Effect of Kaftrio on Perspectives of Health and Wellbeing in Individuals with Cystic Fibrosis. Int J Environ Res Public Health 2022; 19(10).
[4] VanElzakker MB, et al. Neuropsychiatric adverse effects from CFTR modulators deserve a serious research effort. Curr Opin Pulm Med 2023;29(6):603–9.
[5] FDA. Kaftrio information file. 2021. https://www.ema.europa.eu/en/medicines/human/EPAR/kaftrio#authorisation-details-section.
[6] Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 1999;36(2–3):179–94.
[7] Ripani P, et al. Maternal, newborn and breast milk concentrations of elexacaftor/tezacaftor/ivacaftor in a F508del heterozygous woman with cystic fibrosis following successful pregnancy. Front Med (Lausanne) 2023;10.
[8] Taylor-Cousar JL, Jain R. Maternal and fetal outcomes following elexacaftor-tezacaftor-ivacaftor use during pregnancy and lactation. J Cyst Fibros 2021;20(3): 402–6.
[9] A study to investigate the safety of kaftrio® in 2 to 5 year olds with cf, https://www.cysticfibrosis.org.uk/get-involved/clinical-trials/trialstracker/tt002106. 2021.
[10] Liessi N, et al. The combination elexacaftor/tezacaftor/ivacaftor (ETI) modulates the de novo synthethic pathway of ceramides in a genotype-independent manner. J Cyst Fibros 2023.
[11] Karsai G, et al. DEGS1-associated aberrant sphingolipid metabolism impairs nervous system function in humans. J Clin Invest 2019;129(3):1229–39.
[12] Mizutani Y, Kihara A, Igarashi Y. Identification of the human sphingolipid C4-hydroxylase, hDES2, and its up-regulation during keratinocyte differentiation. FEBS Lett 2004;563(1–3):93–7.

[13] Michel C, et al. Characterization of ceramide synthesis. A dihydroceramide desaturase introduces the 4,5-trans-double bond of sphingosine at the level of dihydroceramide. J Biol Chem 1997;272(36):22432–7.
[14] Kaylor JJ, et al. Identification of DES1 as a vitamin A isomerase in Muller glial cells of the retina. Nat Chem Biol 2013;9(1):30–6.
[15] Blackburn NB, et al. Rare DEGS1 variant significantly alters de novo ceramide synthesis pathway. J Lipid Res 2019;60(9):1630–9.
[16] Pant DC, et al. Loss of the sphingolipid desaturase DEGS1 causes hypomyelinating leukodystrophy. J Clin Invest 2019;129(3):1240–56.
[17] Tzou FY, et al. The pathophysiological role of dihydroceramide desaturase in the nervous system. Prog Lipid Res 2023;91:101236.
[18] Dolgin V, et al. DEGS1 variant causes neurological disorder. Eur J Hum Genet 2019;27(11):1668–76.
[19] Poliakov E, et al. Inhibitory effects of fenretinide metabolites N-[4-methoxyphenyl] retinamide (MPR) and 4-oxo-N-(4-hydroxyphenyl)retinamide (3-keto-HPR) on fenretinide molecular targets beta-carotene oxygenase 1, stearoyl-CoA desaturase 1 and dihydroceramide Delta4-desaturase 1. PLoS One 2017;12(4):e0176487.
[20] Casasampere M, et al. Inhibitors of dihydroceramide desaturase 1: therapeutic agents and pharmacological tools to decipher the role of dihydroceramides in cell biology. Chem Phys Lipids 2016;197:33–44.
[21] Guilbault C, et al. Fenretinide corrects newly found ceramide deficiency in cystic fibrosis. Am J Respir Cell Mol Biol 2008;38(1):47–56.
[22] Guilbault C, et al. Cystic fibrosis fatty acid imbalance is linked to ceramide deficiency and corrected by fenretinide. Am J Respir Cell Mol Biol 2009;41(1):100–6.
[23] Thomas JS, et al. A phase I study of intravenous fenretinide (4-HPR) for patients with malignant solid tumors. Cancer Chemother Pharmacol 2021;87(4):525–32.
[24] Pigliasco F, et al. Simultaneous quantification of ivacaftor, tezacaftor, and elexacaftor in cystic fibrosis patients' Plasma by a novel LC-MS/MS method. Biomedicines 2023;11(2).
[25] Fagerberg L, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 2014;13(2):397–406.
[26] Davis DL, et al. Dynamics of sphingolipids and the serine palmitoyltransferase complex in rat oligodendrocytes during myelination. J Lipid Res 2020;61(4):505–22.
[27] Visigalli D, et al. Exploiting sphingo- and glycerophospholipid impairment to select effective drugs and biomarkers for CMT1A. Front Neurol 2020;11:903.
[28] Farrar MA, et al. Evolution of peripheral nerve function in humans: novel insights from motor nerve excitability. J. Physiol. (Lond.) 2012;591(1):273–86.
[29] de Faria O, et al. Periods of synchronized myelin changes shape brain function and plasticity. Nat Neurosci 2021;24(11):1508–21.
[30] Kinney HC, et al. Myelination in the developing human brain: biochemical correlates. Neurochem Res 1994;19(8):983–96.
[31] Doretto S, et al. Oligodendrocytes as Regulators of Neuronal Networks during Early Postnatal Development. PLoS One 2011;6(5).