In Vitro Pharmacological Characterization of TAVT-135, a Novel Chloride Ion Transporter for Pan-Genotypic Treatment of Cystic Fibrosis

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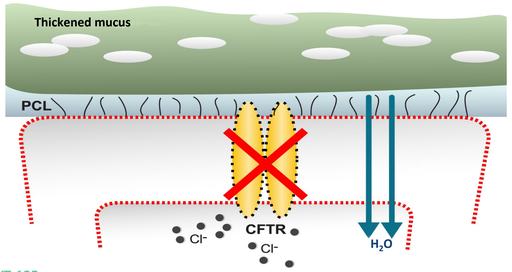
Background

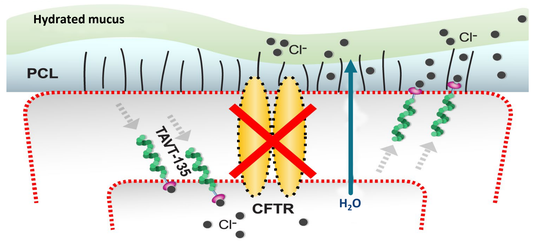
- Modulators of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) have transformed the treatment of CF,[1,2] however:
  - approximately 10% of patients have ineligible genotypes[3]
  - inadequate response or intolerance to CFTR modulators persists[4]
- TAVT-135 is a novel, cell-penetrating peptide (CPP) conjugate that facilitates chloride ion (Cl^-) transport (**Figure 1**), and is being investigated as a potential treatment for CF, irrespective of *CFTR* mutation

Figure 1. Working model of TAVT-135 mechanism of action

a. CF airway



b. CF airway with TAVT-135



CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; PCL, periciliary layer
Chloride ions depicted as black circles and bacterial colonization depicted as gray ovals. Blue arrows indicate the overall direction of water movement

Objective

- To characterize the electrophysiological and mucus-hydrating properties of TAVT-135 through a series of *in vitro* studies

Methods

Intracellular to extracellular chloride-ion transport

- Measured in *Xenopus laevis* oocytes using a two-electrode voltage-clamp technique in the presence of TAVT-135 (10 µM) or negative controls [CPP alone or the chloride-binding moiety alone (both 10 µM)]

Electrophysiological correlates of chloride ions

- Evaluated anion efflux using a modified Ussing chamber system with human bronchial epithelial cells harboring mutations for non-functional CFTR (non-functional genotype with *W1282X/R1162X* mutation)
- The impact of acute and chronic exposure to TAVT-135 on short-circuit current (I_{SC}) was measured following amiloride-induced inhibition of the epithelial sodium channel, and transepithelial electrical resistance (TEER) was also determined
  - Acute TAVT-135: 0.01 to 50 µM
  - Chronic TAVT-135: 1 µM for up to 96 hours, followed by acute TAVT-135 (1 µM)
- Treatment differences for TAVT-135 versus controls were evaluated using a Student's *t* test

Mucus hydration

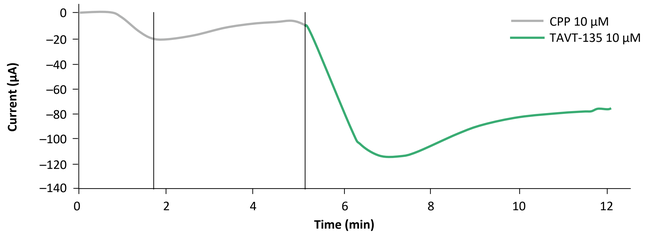
- Measured the height of the airway surface liquid (ASL) and periciliary layer (PCL) in CFBE41o- cells (human CF bronchial epithelial cell line; homozygous for ΔF508)
- Following chronic exposure to TAVT-135 (1, 10, and 100 µM) for 48 hours, ASL and PCL were visualized with apical application of fluorescein isothiocyanate-dextran and heights (µm) were determined using Z-stack images from confocal microscopy

Results

Intracellular to extracellular chloride-ion transport

- In *X. laevis* oocytes, TAVT-135 induced a rapid Cl^- efflux, demonstrating Cl^- transport from the intracellular to the extracellular space
- The CPP alone had minimal activity (**Figure 2**), and the chloride-binding moiety alone, without CPP, had no effect on Cl^- efflux (data not shown)

Figure 2. Effect of CPP (negative control) and TAVT-135 on chloride ion currents in *Xenopus* oocytes



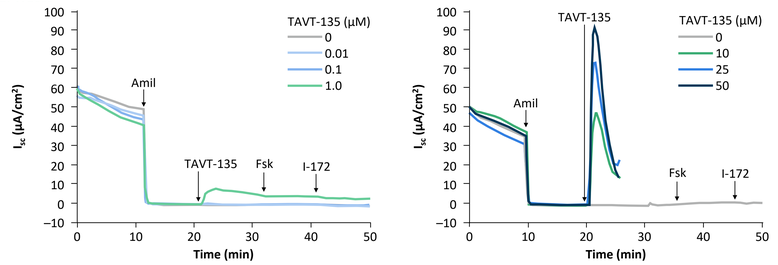
CPP, cell-penetrating peptide

Electrophysiological correlates of chloride ions

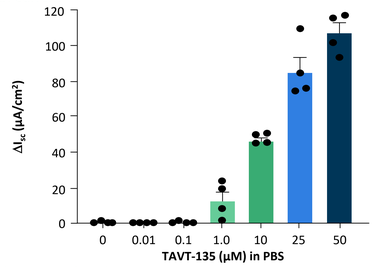
- In human bronchial epithelial cells, there was a significant, dose-dependent increase in I_{SC} following acute application of TAVT-135 ≥1 µM, demonstrating anion efflux (**Figures 3a and 3b**)
- Within 5 minutes of acute exposure, TEER was maintained at concentrations ≤1 µM and decreased at concentrations ≥10 µM (**Figure 3c**)
- After chronic exposure for up to 96 hours, acute application of TAVT-135 (1 µM) increased I_{SC} (**Figure 3d**), while TEER was maintained (**Figure 3e**)

Figure 3. Electrophysiological effects of TAVT-135 in primary airway cultures following acute application (a–c) and chronic (96-hour) application (d and e)

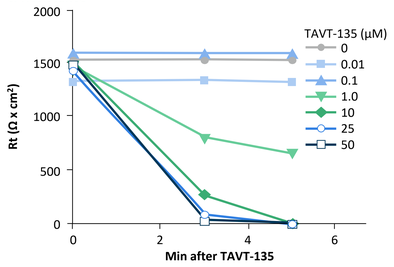
a. Traces



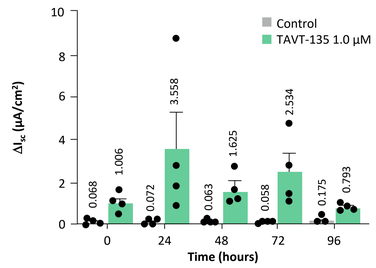
b. Current



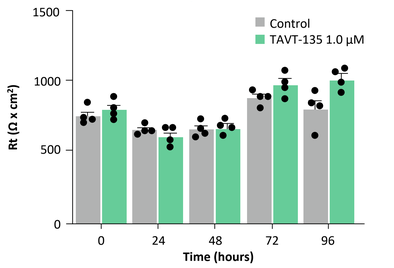
c. Resistance



d. Current after chronic exposure



e. Resistance after chronic exposure



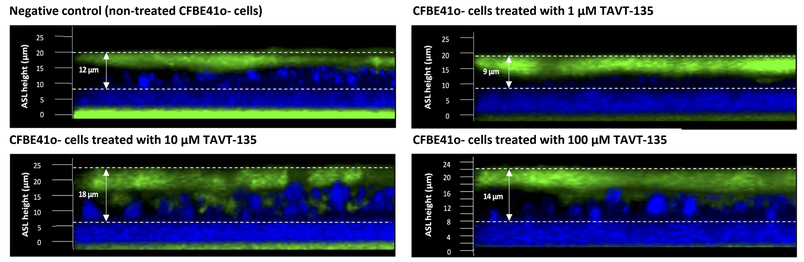
Amil, amiloride; fsk, forskolin; I_{SC}, short-circuit current; PBS, phosphate-buffered saline; Rt, transepithelial resistance
Black dots are individual measurements; bars are mean ± standard error of the mean

Mucus hydration

- After 48 hours of incubation, TAVT-135 (10 and 100 µM) significantly increased ASL and PCL height in human CF bronchial epithelial cells with *CFTR* mutations compared with the untreated control (**Figure 4**)

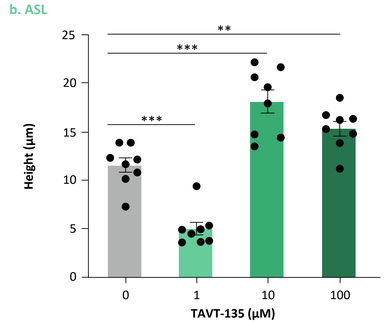
Figure 4. Effect of TAVT-135 on ASL and PCL height in *CFTR*-mutated human CF bronchial epithelial cell line

a. Representative Images

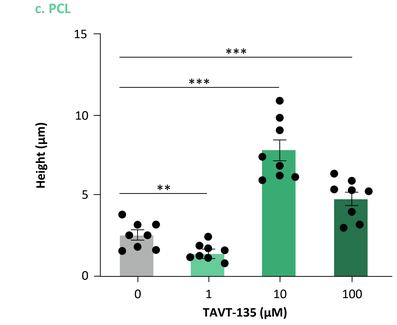


Green: Fluorescein isothiocyanate-dextran (4 kDa) Blue: Hoechst 33342
Objective: 20x

b. ASL



c. PCL



ASL, airway surface liquid; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; PCL, periciliary layer
**p<0.01; ***p<0.001
Black dots are individual samples; bars are mean ± standard error of the mean

Conclusions

- Collectively, these data showed that TAVT-135:
  - Rapidly induced intracellular Cl^- transport across plasma membranes without negatively impacting the epithelial barrier
  - Increased ASL and PCL height, suggesting the potential for a mucus-hydrating effect *in vivo*
- TAVT-135 has the potential to address significant unmet needs in patients with CF, including those who are ineligible for or not responding to CFTR modulators
- Additional studies are ongoing to further characterize the mechanism of action

References

1. Despotes KA, Donaldson SH. Curr Opin Pharmacol 2022;65:102239.
2. Regard L et al. Cells 2022;11:1769.
3. Fajac I, Sermet I. Cells 2021;10:2793.
4. Guimbellot J et al. Pediatr Pulmonol 2017;52(Suppl 48):S4–S14.

Disclosures

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