Highway to Cell: Internalization of CFTR–Stabilizing Peptides as Potent Cystic Fibrosis Treatment

https://doi.org/10.17952/37EPS.2024.P2127

prisca.boisguerin@inserm.fr

Clémentine Teko-Agbo[(1)], Emilie Josse[(1)], Quentin Seisel[(2)], Dean R. Madden[(3)], Magali Taulan Cadars[(1)], Prisca Boisguérin[(1)]

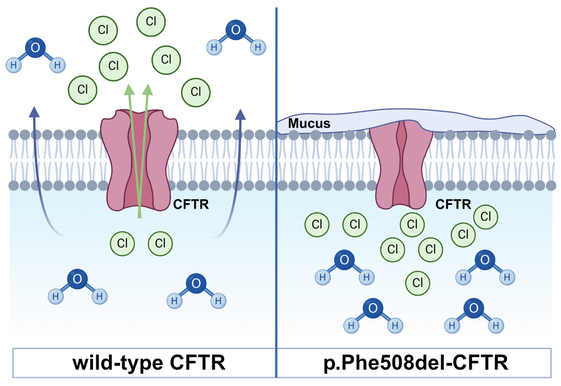
(1) PhyMedExp, University of Montpellier, INSERM U1046, CNRS UMR 9214, 34295 Montpellier, France (2) University of Montpellier, 34090 Montpellier, France
(3) Geisel School of Medicine at Dartmouth, 7200 Vail Building, Hanover, NH 03755-3844, USA

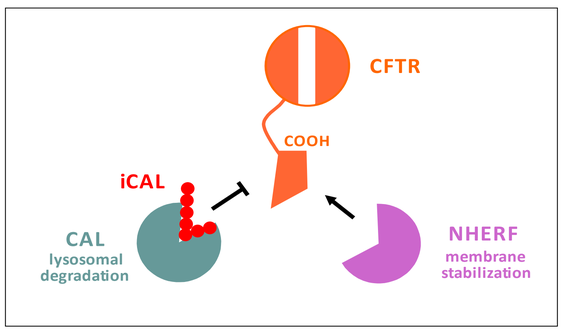
Abstract

Cystic fibrosis (CF) is due to loss-of-function mutations in the CFTR chloride channel. The consequences on the airways are:
- impairment of mucociliary clearance
- higher prevalence of chronic bacterial infections [1]

One of the three known functional defects associated with the most prevalent mutation, p.Phe508del-CFTR, concerns the stability of this chloride channel at the apical membrane of endothelial cells. Previously, our group has reported the development of CAL inhibiting peptides (iCAL36) which rescue CFTR activity (11% increase in chloride efflux) [4, 5].

Indeed, both wt- and p.Phe508del-CFTR can be degraded through a lysosomal pathway involving the CFTR:CAL protein-protein interaction [2], hence reducing its lifetime. In contrast, NHERF1/2 proteins anchor the CFTR channel at the apical membrane of epithelial cells.
Thus, our strategy thus consists of the development of an interfering iCAL peptide targeting specifically the CAL protein. As a consequence of the CFTR:CAL inhibition, CFTR rescuing at the apical cell membrane should be observed resulting in increased physiological functionality.
To improve iCAL activity, the presented work concerns the optimization of the iCAL36 interfering peptide via different cell-penetrating peptides (CPP) [6] as a delivery system.





CONCLUSION

Interfering iCAL peptide as CFTR stabilizer

- Cell-penetrating peptides for iCAL36 internalization
- Penetratin showing optimal internalization
- Internalization involve endocytosis-dependent pathways
- TRI-iCAL36 as optimal peptide for cell internalization
- TRI-iCAL36 increase CFTR amount (accumulation)
- But needs a VX pre-incubation

I – Improvement of iCAL36 cellular delivery

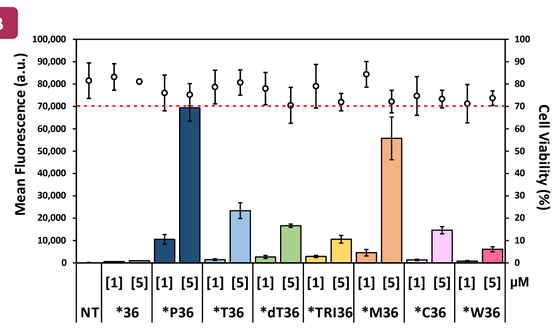
Cell-penetrating peptides (CPP) can cross biological membranes, alone or while being associated to another molecule called cargo. We selected seven CPP (MPG & Penetratin [9],Tat [10], its D- and retro-inverso isoform [11], C6M1 [12] and WRAP5 [13] to improve cellular internalization and thus biological activity of the iCAL36 peptide. Resulting CPP-iCAL36 conjugates were evaluated in terms of their cellular internalization on epithelial cells (Caco-2) expressing wt-CFTR as a model [14].

FIGURE 1

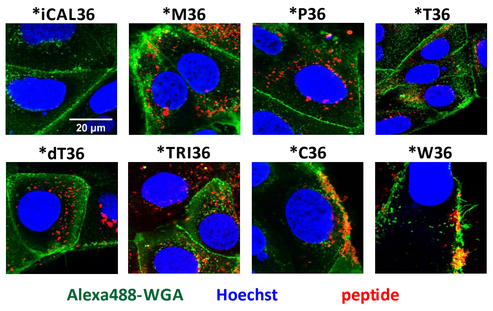
A

Name	Structure	Residues
iCAL36	ANSRWPTSII	10
MPG-iCAL36	GALFLGWLGAAGSTMGAWSQPKKKRKV-ANSRWPTSII	37
Pen-iCAL36	RQILIWFQNRRMKWKK-ANSRWPTSII	26
Tat-iCAL36	GRKKRRQRRRPPQ-ANSRWPTSII	23
dTat-iCAL36	grkkrrqrrrppq-ANSRWPTSII	23
TRI-iCAL36	qpprrrqrrkkrg-ANSRWPTSII	23
C6M1-iCAL36	RLWRLLWRLWRRLWRLL-ANSRWPTSII	28
WRAP5-iCAL36	LLRLLRWWWRLLRLL-ANSRWPTSII	25

B



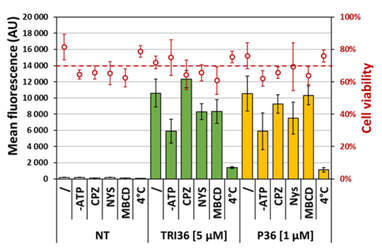
C



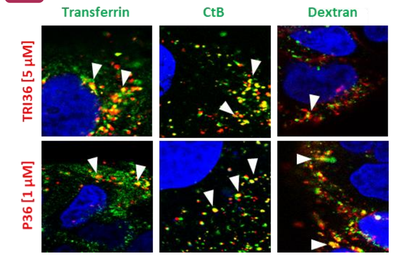
Internalization properties are assayed by flow cytometry and by confocal microscopy using TAMRA-labelled peptides (denoted by *) on Caco-2 cells. **Left panel:** Cells were incubated with TAMRA-CPP-iCAL36 and mean fluorescence values corresponding to the internalized peptides were quantified by flow cytometry. DAPI staining allow the acquisition of the cell viability. **Right panel:** Representative images of TAMRA-CPP-iCAL36 cellular distribution in living Caco-2 cells. Co-staining with Hoechst (blue, nucleus) and Alexa488-WGA (green, cell membrane) were performed for better visualization. Both secondary amphipathic peptides (C36 and W36) are mainly sticking at the cell membrane. All other CPP-iCAL conjugates are visible as punctuated patterns in the cytosol which could be an indication for an endocytosis-dependent internalization.

FIGURE 2

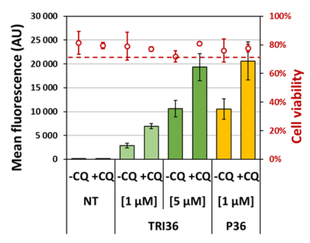
A



B



C



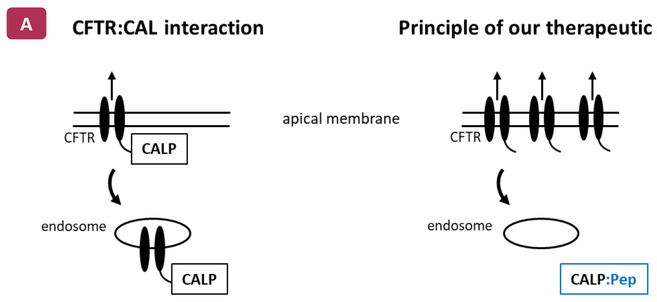
Left panel: Cells were incubated with the peptide at 4°C or co-incubated with endocytosis inhibitors (-ATP = NaN3/DG, CPZ = Chlorpromazine, NYS = Nystatin, MbCD = methyl-beta-cyclodextrin) and compared to the standard condition (37°C). Two conditions induced an internalization reduction (~85% for 4°C and ~45% for ATP depletion) suggesting an energy-dependent internalization. **Middle panel:** Representative images of TAMRA-labelled peptides co-transfected with endocytosis markers (Transferrin, Cholera toxin, and Dextran - in green) in living Caco-2 cells. Yellow staining revealed a colocalization of the peptide with the markers. Cholera toxin colocalization seems to be unspecific. White bar = 20 µm. **Right panel:** Cells were incubated with the peptide alone or in the presence of chloroquine (CQ) an endosomolytic reagent. Increased fluorescence level after CQ treatment suggests an endosomal encapsulation of the peptide.

II — TRI–iCAL36 increase CFTR amount

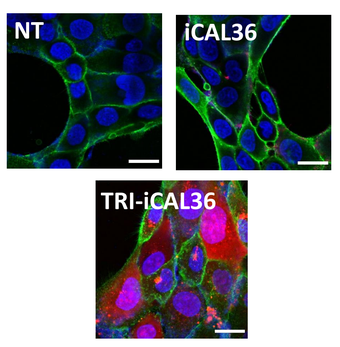
The stabilizing property of TatRI-iCAL36 was evaluated indirectly. Because the peptide might inhibit the interaction of the CAL protein with the C-terminus of the mutated p.Phe508del-CFTR, CFTR degradation via the endosomal–lysosomal pathway should be reduced or abolished. As a consequence, an increase in the total CFTR amount should be detected in the 16HBEge cells.
Indeed, for the first time, we were able to reveal in p.Phe508del-CFTR 16HBE epithelial cells an additive increase in the CFTR amount in the presence of VX-445/VX-809 compared to VX-445/VX-809 treatment alone.

FIGURE 3

A



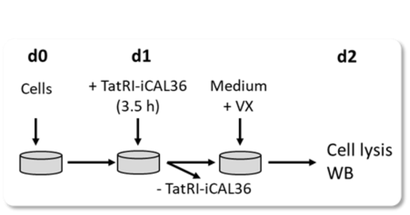
B



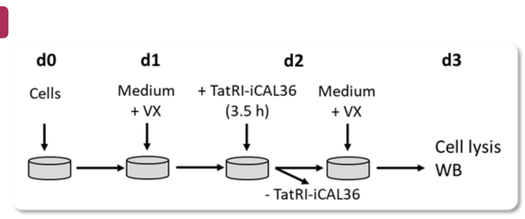
Left panel: Scheme highlighting the activity of the interfering iCAL peptide. **Right panel:** Representative images of living 16HBEge cells imaged by CLSM after 3 h incubation with Tamra-iCAL36 (10 µM) or Tamra-TRI-iCAL36 (10 µM)) in OptiMEM. Nuclei are labeled with Hoechst dye. Bars represent 10 µm.

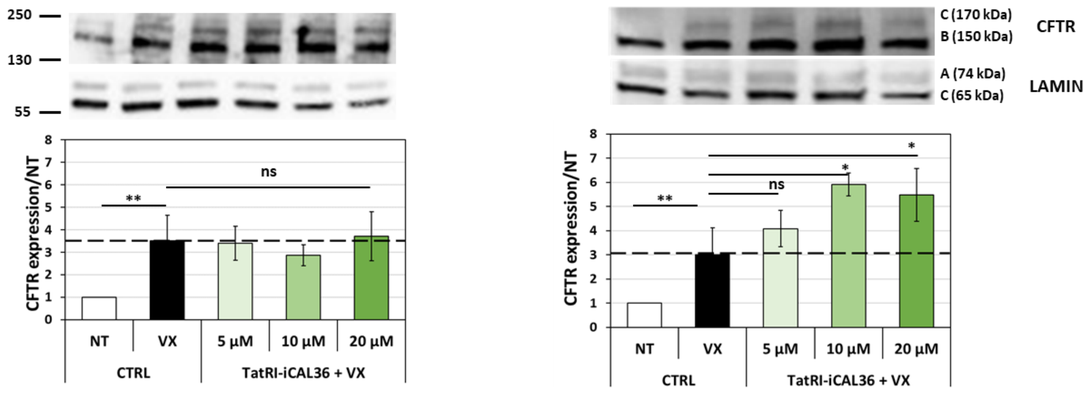
FIGURE 4

A



B





Activity of TatRI-iCAL36 in 16HBEge cells. Left panel: 16HBEge cells were incubated with TatRI-iCAL36 (5 µM, 10 µM, or 20 µM) in the presence of the corrector VX-445/VX-809 cocktail as indicated in the scheme. On day 2 (d2), the cells were lysed and p.Phe508del-CFTR was quantified by Western blot. **Right panel:** 16HBEge cells were pre-incubated with the correction VX-445/VX-809 for 24 h. Afterward, the cells were incubated with TatRI-iCAL36 (5 µM, 10 µM, or 20 µM) in the presence of the VX-445/VX-809 corrector cocktail as indicated in the scheme. On day 3 (d3), cells were lysed and p.Phe508del-CFTR was quantified by Western blot.

References

[1] Kerem et al., Science, 245, 1073 (1989). - [2] Wolde et al., J Biol Chem, 282, 8099 (2007). - [3] Vouilleme et al., Angew Chem Int Ed Engl, 49, 9912 (2010). - [4] Cushing et al., Angew Chem Int Ed Engl, 49, 9907 (2010). - [5] Frank, J Immunol Methods, 267, 13 (2002). - [6] Heitz et al., Br J Pharmacol, 157, 195 (2009). - [7] Boisguerin et al., Chem Bio Chem, 8, 2302 (2007). - [8] Seisel et al., Bioorg Med Chem Lett, 27, 3111 (2017). - [9] Müller et al., J Pep Sci, 18, 293 (2012). - [10] Vivès et al., J Biol Chem, 272, 16010 (1997). - [11] Meloni et al., Cell Mol Neurobiol, 34, 173 (2014). - [12] Jafari et al., PLoS ONE, 9, e97797 (2014). - [13] Konate et al., Bioconjug Chem, 30(3), 592 (2019). - [14] Seisel et al., Pharmaceitics, 14(4), 808 (2022).

Financial support

anr agence nationale de la recherche AU SERVICE DE LA SCIENCE
RECHERCHE EN SANTÉ RESPIRATOIRE FONDS DE DOTATION
VAINCRE LA MUCOVISCIDOSE