**Abstract**

Potentiators may be necessary for any combination therapy for ΔF508 CF treatment. Potentiators such as ivacaftor (VX-770) and genistein increase the open probability of ΔF508-CFTR when applied acutely. However, when used as drugs, cells will be exposed to potentiators for long periods of time. We investigated the long-term effect of potentiators such as VX-770, genistein and FD2033129 (a potentiator discovered and under development at Flatley Discovery Lab (FDL)) on function and surface expression of ΔF508-CFTR.

As revealed by equivalent current measurements, we found that chronic (24 h) exposure of primary ΔF508 homozygous hBE cells to VX-770 reduced ΔF508-CFTR mediated Cl currents. Dose response curves of the ΔF508-CFTR corrector VX-809 in the absence and presence of 1 µM VX-770 reveal an average 50% reduction in ΔF508-CFTR Cl currents (n=30), when compared with acutely potentiated cells, at all doses of VX-809. We observed similar results in cells treated with ΔF508-CFTR correctors VX-661 or FDL304. The EC50 for each corrector did not change in the presence of chronic VX-770.

Dose inhibition curves were obtained with all 3 potentiators and DMSO control. VX-770 induced a maximum ∼66% reduction in current at the highest doses (n=8). VX-770 inhibited the ΔF508-CFTR Cl currents with an IC50=2.5 nM (N=4) regardless of whether the CFTR was corrected by VX-809 or not, and this is the same EC50 we measure for the acute potentiation of forskolin stimulated ΔF508-CFTR in primary human airway cells. In uncorrected cells, reduction of CFTR current reached 100% with VX-770. Chronic exposure of FD2033129 on VX-809 or FD304 corrected or on uncorrected cells (DMSO) showed no reduction of CFTR currents. Chronic genistein dose inhibition curves are similar to that seen by A. Schmidt, et al. (Br J Pharmacol. 2008;153:1311-23). In corrected (or their WT) cells, there is a slight increase in current at about 3 µM and a strong inhibition at 30 µM (n=8), due to genistein inhibition of CFTR. The increase at 3 µM was not seen with uncorrected CF cells. We tested prolonged exposure of FD2033129 and VX-770 on cell surface expression in CBE140- cells. ΔF508-CFBE cells corrected with VX-809 and with or without chronic exposure to FD2033129 shows no significant difference in cell surface expression of CFTR (n=16). Whereas, ΔF505-CFBE cells corrected with VX-809 and with chronic exposure to VX-770 shows 60% reduction in cell surface CFTR expression (n=16). In uncorrected cells, there is expression of some ΔF508 CFTR and this was completely eliminated by 5 µM VX-770, just as seen in electrophysiology. When tested in the cell surface HRP assay with ΔF508-R1070W-CFTR (n=4) and ΔF505-I539T-CFTR (n=4) background, there was no chronic inhibition effect of potentiators VX-770 or FD2033129.

**Conclusion**

Prolonged exposure to VX-770 reduced the ΔF508-CFTR current in primary cells, whereas prolonged exposure to FD2033129 did not. The chronic effect of VX-770 is independent of corrector co-treatment. These results were also confirmed in cell surface HRP assays. These data indicate that VX-770 and a corrector would not be an optimal combination for ΔF508-CFTR CF patients and chronic inhibition is not a general property of potentiators.

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