49 Rescue of the protein folding defect in cystic fibrosis *in vitro* by the investigational small molecule, VX-809

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Despite the discovery of the cystic fibrosis (CF) gene in 1989, there are no approved therapies that target the underlying defect in the CF gene product, the CF transmembrane conductance regulator (CFTR). Ninety percent of CF patients carry the F508del CFTR mutation that leads to defects in CFTR protein folding, preventing it from reaching the cell surface where it normally regulates salt transport and water movement across epithelial cells. Here, we describe the pharmacology of VX-809, an investigational small molecule CFTR corrector that increased the cell surface density of F508del-CFTR *in vitro*. In these studies, we show that VX-809 increased the amount of properly folded CFTR that was able to exit from the endoplasmic reticulum and, as a result, increased anion transport across human CF airway cell cultures carrying the F508del-CFTR mutation on both alleles from 3% to approximately 15% of values observed in non-CF airway. This increase was sufficient to augment fluid secretion from these airway cultures, which could be further improved by the addition of VX-770, a CFTR potentiator. These *in vitro* studies suggest that small molecule drugs such as VX-809, either alone or in combination, may directly address the underlying genetic defect in CF to restore CFTR function.

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50* The investigational CFTR potentiator, VX-770, potentiated multiple CFTR forms in vitro

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Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductor (CFTR) gene, which encodes a PKA-activated anion channel. Increasing Cl^- ion flow through cell-surface CFTR is a potential therapeutic approach in CF. Previous *in vitro* studies showed that the investigational CFTR potentiator, VX-770, increased the gating activity of the G551D (class III gating mutation) and F508del (class II trafficking mutation) forms of CFTR. This *in vitro* study aimed to evaluate if VX-770 can potentiate the activity of other CFTR mutant forms. We measured the effect of VX-770 on CFTR-mediated Cl^- transport in recombinant cells expressing the 50 most common missense and deletion *CFTR* mutations. Class III mutations demonstrated the largest VX-770 response, with all 8 class III mutations tested (e.g., G178R, G551D, G551S, G1349D) showing a >10-fold increase in Cl^- transport compared to baseline. VX-770 also potentiated, albeit to a lesser extent, class IV (conductance) mutations (e.g., R117H, D1152H, I148T), class II mutations that result in a small amount of CFTR trafficking to the cell surface (e.g., F508del, S549R), and multiple unclassified CFTR mutant forms. For the majority of class II mutations other than F508del and S549R, no response to VX-770 was observed in the absence of compounds that correct defective CFTR trafficking. VX-770 also increased Cl^- transport in cultured human bronchial epithelia from CF patients carrying the F508del mutation and a class V (reduced synthesis; e.g., 2789+5G>A, 3272–26A>AG) mutation on the other allele. These *in vitro* data support the rationale for evaluating VX-770 monotherapy in CF patients carrying a variety of CFTR mutant forms.

51 How can the CFTR potentiator CBIQ activate the calcium activated K^+ channel KCa3.1?

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In the last years, several small molecules were identified by high throughput screening that could restore mutated CFTR function. Compounds addressing CFTR gating defects are referred to as potentiators. The basolateral K^+ channel KCa3.1 has been documented to play a prominent role in establishing a suitable driving force for CFTR-mediated Cl^- secretion in airway epithelial cells. Thus, a research aimed at identifying structural parameters by which CFTR potentiators can simultaneously activate both CFTR and KCa3.1 offers new perspectives to the correction of ion transport defects in CF epithelia. Patch clamp measurements, computer modeling and site directed mutagenesis approaches were thus used to characterize the molecular determinants underlying the action of CBIQ a CFTR potentiator documented to also activate KCa3.1. Our results show that CBIQ destabilizes a non conducting state of the channel by binding to a site accessible irrespectively of the channel conducting state. Experiments carried with the constitutively active A279G mutant also confirmed that Ca^{2+} is absolutely required to the action of CBIQ. Experiments in which Ba^{2+} was used as a probe were finally performed to determine if the action of CBIQ involves an effect on the selectivity filter. Our results showed that Ba^{2+} can displace CBIQ from its activity site, suggesting that the increases in channel activity induced by CBIQ could result from a change in the energetic of the channel at the level of the selectivity filter. On the basis of our results, we conclude that CBIQ, a CFTR potentiator, can activate KCa3.1 by binding to a hydrophobic pocket connected to the channel selectivity filter region.

52* CFTR missing the last four transmembrane segments and nucleotide binding domain 2 resides in the ER but escapes the cell's quality control mechanism

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CFTR is composed of two transmembrane domains, two nucleotide domains and a regulatory domain. In the kidney a truncated splice variant of CFTR is present with 145 bases pairs deleted that results in a frame shift and a premature stop codon leading to a new protein that includes only the first half of CFTR called TNR CFTR. The purpose of this study is to understand the trafficking, processing of TNR and why the kidney is not involved in Cystic Fibrosis.

TNR is easily detectable as two distinct bands. When cells were transfected with TNR and treated for 16 hours with proteasome inhibitors, the protein expression was not significantly changed. A similar response was also observed with lysosomal inhibitors. These data suggest that TNR protein is not recognized by the cell's quality control mechanism as a mutant protein. To evaluate the rate of degradation cells were treated with cycloheximide. The results showed that TNR protein has a similar time course of degradation compared to wt CFTR. Surface biotinylation studies show that only a small fraction of TNR resides at the plasma membrane. Also TNR cannot act to transcomplement ΔF508 CFTR in contrast to other truncated CFTR such as Δ264 which is missing the N-terminal portions of CFTR. Consistent with these data is that small molecule correctors such as 4A and VRT325 which do promote ΔF508 CFTR trafficking out of the ER do not affect TNR CFTR. Our data suggest that TNR is a very stable protein, the majority of which resides within the ER. This can be compared to Δ264 and ΔF508 CFTR both are recognized as mutant proteins and rapidly degraded.

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