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Original Article

Combination potentiator ('co-potentiator') therapy for CF caused by CFTR mutants, including N1303K, that are poorly responsive to single potentiators

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Abstract

Background: Current modulator therapies for some cystic fibrosis-causing CFTR mutants, including N1303K, have limited efficacy. We provide evidence here to support combination potentiator (co-potentiator) therapy for mutant CFTRs that are poorly responsive to single potentiators.
Methods: Functional synergy screens done on N1303K and W1282X CFTR, in which small molecules were tested with VX-770, identified arylsulfonamide-pyrrolopyridine, phenoxy-benzimidazole and flavone co-potentiators.
Results: A previously identified arylsulfonamide-pyrrolopyridine co-potentiator (ASP-11) added with VX-770 increased N1303K-CFTR current 7-fold more than VX-770 alone. ASP-11 increased by ~65% of the current of G551D-CFTR compared to VX-770, was additive with VX-770 on F508del-CFTR, and activated wild-type CFTR in the absence of a cAMP agonist. ASP-11 efficacy with VX-770 was demonstrated in primary CF human airway cell cultures having N1303K, W1282X and G551D CFTR mutations. Structure-activity studies on 11 synthesized ASP-11 analogs produced compounds with EC_{50} down to 0.5 μM.
Conclusions: These studies support combination potentiator therapy for CF caused by some CFTR mutations that are not effectively treated by single potentiators.

Keywords: Cystic fibrosis; CFTR; High-throughput screen; Potentiator; N1303K

1. Introduction

Cystic fibrosis is caused by loss of function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR is a cAMP-regulated, ATP-gated chloride channel that is normally expressed at the apical plasma membrane of epithelial cells in the airways, intestine, pancreatic duct and other tissues [1]. There has been remarkable progress in the development of drugs to treat the underlying cellular processing and gating defects produced by mutations in CFTR. The potentiator ivacaftor (VX-770) has been approved to treat cystic fibrosis caused by the G551D mutation and at least 38

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other mutant CFTRs with defective channel gating [1]. The corrector lumacaftor (VX-809) in combination with ivacaftor has been approved to treat cystic fibrosis caused by the F508del mutation [1]. However, the limited efficacy of lumacaftor/ivacaftor therapy in cell models and human clinical trials has motivated the development of corrector combination therapies in which a potentiator is combined with two correctors, each in principle targeting a distinct structural or dynamic defect in F508del-CFTR [2]. We previously reported the first 'corrector synergy' screen to identify compounds, which when added together with lumacaftor, produced greater efficacy than maximal lumacaftor alone [3].

While approved and investigational potentiators and correctors, when sufficiently advanced, may be beneficial to most patients with cystic fibrosis, there remains ~10% of patients that are not amenable to these therapies [1]. The most common CFTR mutations in these subjects include premature stop codon (PTC) mutations such as G542X and W1282X, and point mutations such as N1303K, which appear to be refractory to available potentiators and correctors. N1303K-CFTR is a missense Class II mutation, with defective CFTR processing, cell-surface trafficking, and channel gating [1,4–6]. Globally, of the ~88,000 CF subjects that have provided information about their CFTR mutations in the CFTR2 database, 99 (~0.1%) and 1238 (~1.4%) have N1303K/N1303K and N1303K/F508del CFTR mutations, respectively. CFTR modulator triple combinations may be applicable to some homozygous CF subjects with F508del and a 'minimal function mutation', including N1303K/F508del and W1282X/F508del. Clinical development of these therapies is presently at phase II and soon to enter phase III trials.

Here, we advance the concept of combination potentiator ('co-potentiator') therapy for cystic fibrosis caused by difficult-to-treat CFTR mutations that appear to be refractory to treatment by single potentiators alone or in combination with correctors. Co-potentiators identified here from 'potentiator synergy' screens were found to act in synergy or additively when used in combination with ivacaftor, for the refractory CFTR mutants N1303K and W1282X, as well as for F508del and G551D. This work was motivated by our prior studies on the truncated protein product produced by the W1282X mutation [7], in which a potentiator in combination with VX-770 increased W1282X-CFTR function about 8-fold over that of VX-770 alone, normalizing W1282X-CFTR channel activity to that of wild-type CFTR.

2. Results

2.1. N1303K-CFTR cell line generation and potentiator screen

FRT cells stably expressing N1303K-CFTR and the halide sensor YFP-H148Q/I152L/F46L (FRT-N1303K-YFP) were generated for screening, in a similar manner as previously described for wild-type CFTR and CFTR mutants F508del, G551D and W1282X [7,8]. As indicated in Fig. 1A (top), the N1303 residue is located in the nucleotide binding domain 2 (NBD2) of the CFTR protein. Fig. 1A (bottom) shows a fluorescence image of transfected cells expressing N1303K-CFTR and the YFP sensor. The basis of our screening strategy is shown in Fig. 1B. For a potentiator screen, following 24-h incubation with a corrector to partially rescue N1303K-CFTR cellular misprocessing, a test compound, together with a high concentration of forskolin, was added 10 min prior to assay. The functional assay involved measurement of YFP fluorescence quenching following addition of iodide to the extracellular solution.

A panel of known correctors was first tested in the FRT-N1303K-YFP cells to identify compounds suitable for use in screening to identify potentiators. Correctors tested included: VX-809, VX-661, C3 and C18, which target the NBD1-MSDs interface; C4, which targets NBD2-MSDs; CoPo-22, a CFTR modulator with dual corrector and potentiator function; $W1282X_{corr}$-A23, which corrects the protein product generated by the W1282X-CFTR nonsense mutation; and sodium butyrate, a known transcriptional activator for the CMV promoter [3,7,9]. Fig. 1C shows representative YFP fluorescence quenching data (left panel) and a data summary for tested correctors (right panel). Little or no corrector activity was seen for most of the compounds, with only sodium butyrate and C3 providing significant correction. Sodium butyrate, which was used for screening here because of limited C3 availability, is likely acting as a transcriptional activator in this transfected cell system, resulting in increased cell surface expression. In addition, a panel of known potentiators was tested in the FRT-N1303K-YFP cells (Fig. 1D). Of the known potentiators, VX-770 increased channel gating activity whereas PG-01 (P2), A04 and CoPo-22 were inactive. Taken together, the data show that N1303K-CFTR does not respond well to available correctors and potentiators.

Short-circuit current was measured in the N1303K-CFTR-expressing FRT cells to validate the data obtained by the fluorescence plate reader assay, and as a quantitative measure of CFTR chloride current. Measurements were done following permeabilization of the basolateral cell membrane with amphotericin B and in the presence of a transepithelial chloride concentration gradient so that current provides a linear measure of CFTR chloride current. Without corrector, the FRT cells expressing N1303K-CFTR showed a small chloride current following addition of maximal forskolin and 5 μM VX-770, which was inhibited by $CFTR_{inh}$-172 (Fig. 1E); correction by 3 μM C3 or 3 mM sodium butyrate increased short-circuit current by 2–3-fold, in accord with the plate reader data.

In an initial plate reader-based potentiator screen, testing of 9600 synthetic small molecules identified six weakly active compounds; however, none of these compounds showed potentiator activity, even at high concentration (25 μM), when confirmatory short-circuit current analysis was performed. Motivated by prior experience with the W1282X nonsense CFTR mutation in which we discovered that the arylsulfonamide-pyrrolopyridine ASP-11 (previously named $W1282X_{pot}$-A15; Fig. 1F) acted in synergy with VX-770 to restore W1282X mutant channel gating to near wild-type CFTR activity [7], we performed a potentiator synergy ('co-potentiator') screen.

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