VIP-ELP FUSION MOLECULES PB1120 AND PB1046
CORRECT F508DEL-CFTR DYSFUNCTION

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Introduction

Rescuing highly functional F508del-CFTR in CF epithelial cells by correcting its misfolding to promote membrane targeting while increasing surface stability is the major goal of current therapeutic strategies using corrector molecules. We previously reported the importance of prolonged exposure of airway epithelial cells to VIP (Vasoactive Intestinal Peptide), a 28-amino acid neuropeptide released from intrinsic neurons which controls multiple functions in excrine tissues including inflammation, relaxation of airway and vascular smooth muscles and CFTR-dependent secretions.

VIP increases CFTR membrane insertion, stability and function in human airway epithelial cells (Fig. I). Moreover, it corrects the molecular defects associated with the F508del mutation

(Fig. II). With VIP-knockout C57Bl/6 mice, a proven model of airway diseases, we have demonstrated in vivo that the absence of functional VIP can be corrected by exogenous VIP administration (Alcaldé et al. 2014. Am J Physiol–Cell Physiol. 307, C597-209). These results led to the potential of VIP for the treatment of CF.

PhaseBio Pharmaceuticals has developed two VIP-ELP fusion proteins (Fig. III & IV): PB1120, which has similar potency to native peptide for both VAPAC and VAPAC2 receptors, and PB1046 (Yasumura®), which is relatively selective for VAPAC2. PB1046 was well tolerated in 2 phase I clinical trials in subjects with hyperpotassemia and provided exposure of at least 1 week following a single subcutaneous injection (Fig. V).

Here we report F508del-CFTR corrector effects of PB1120 and PB1046 that are highly relevant to the peptides that normally rapidly degrade VIP in vivo and preclude its use as a therapeutic.

Results

Isolated epithelial cells are incubated with 156 mM NaI loading buffer for 1 h. Extracellular NaI solution is then removed and replaced with efflux buffer in which NaI is replaced with NADP. Samples are taken and replaced at 1 minute intervals. The first 3 samples (time 0-2 min) are used to establish a baseline of efflux. CFTR-activity cocktail (cAMP + IBMX + Fg) is included in the efflux buffer from time 3 min. NaI concentration is then measured using an iodine sensitive electrode moved over each sample to a commercial autoanalyzer (Efflux Analysis Software v0.1: Chappe & V. Chappe). NaI efflux rate constant (k) is calculated. Iodoamine peaks (minimum efflux rates during stimulation = basal level) are compared.

Immunoblotting: Membrane proteins are solubilized in RIPA buffer and 100μg of proteins subjected to 6-7.5% SDS-PAGE transferred to PVDF membranes and probed with Monoclonal anti-CFTR antibody.

Immunocytochemistry: MIECT1 cells plated on glass coverslips in low density and maintained in culture at 27°C or 37°C for 24 to 48 hrs were used. Cells were fixed in 2% PFA. Permeabilized with 0.1% Triton X-100% BSA PBS. Primary AQUA2, secondary, goat anti-mouse conjugated to Cy3. Negative controls were performed by omitting the primary antibody.

Statistics: Results are reported as the means ± S.E.M. for 3-5 independent experiments. *p<0.05, **p<0.01, and ***p<0.001.

Conclusions

Our data indicate that PB1120 and PB1046 are strong candidate drugs for the treatment of CF patients with the F508del mutation.

> Prolonged stimulation of human nasal CF cells with VIP-ELP corrects F508del-CFTR trafficking and function better than native VIP or other corrector treatment alone.

> PB1046 1μM 18 hrs alone produces the best functional correction.

> Combining PB1120 with PB1046 with VX770 or VX809 produces an additive positive effect on F508del-CFTR function.

PhaseBio Pharmaceuticals proprietary platform technology uses elastin-like polypeptides (ELPs) genetically fused to peptides and proteins in order to modulate both the rate of release of the therapeutic from the subcutaneous injection site and its half-life in circulation. In addition to this unique dual mechanism of enhancing pharmacokinetics, the ELP platform also provides several other distinct advantages over traditional half-life extension technologies:

> ELP fusions are expressed as soluble proteins in E. coli, allowing for production at high yields and for facile purification.

> ELP fusions are stable in near physiological aqueous buffers, which allows for pharmaceutically elegant, “ready-to-use” formulations (no reconstitution or mixing required).

> The company has had success in co-formulating combinations of ELP fusion versions of proteins that have historically been difficult to combine.

> In data, over 400 clinical trial participants have been exposed to one or more doses of ELP fusion proteins across three development programs (~75% exposed for 20 weeks).

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Figure I. VIP-dependent increase in CFTR membrane density involves two mechanisms:

1. Dissociation of CFTR from CAL in the cytoplasm to promote membrane CFTR insertion

2. Activation of the PKC signaling cascade that promotes NHERF1 / P-ERK complex interaction with membrane CFTR to mediate its surface stability

Figure II. Correction of F508del-CFTR

Membrane and nuclear expression of F508del-CFTR (here illustrated with immunofluorescence and immunoblotting data) were corrected by prolonged VIP treatment in human nasal epithelial cells (MEICFTR) and involved PKC and PKA signaling

Figure III. Preparation of ELP fusion proteins.

(A) Elastin-Like Polypeptides (ELPs) individual subunit, derived from a five amino acid motif found in the human protein elastin, is repeated multiple times to form the ELP heptamer (VPAC). The gene sequence of the therapeutic peptide or protein (in green) is fused at the level of DNA to the gene encoding the heptapetide (in blue) and is expressed as one common fusion protein. The resulting fusion protein retains the activity of the therapeutic domain but assumes the solubility, stability and long half-life of the ELP. These ELP fusion protein can bind and activate the relevant receptor as intact molecules and can be dependent on a drug release mechanism.

Figure IV. Flat PK profile: A unique dual mechanism of slow absorption and extended half-life.

A unique attribute that distinguishes PhaseBio’s technology is the property of ELP fusion molecules to undergo a fully reversible phase transition (or conversion) to form a material that provides a depot for sustained release. Phase transition is driven by thermodynamic forces involving highly recordable micellar hydrophobic and hydrogen bonding interactions.

Figure V. Slow absorption & long half-life.

Fusion to ELPs significantly improves the solubility, stability and bioavailability of peptides and proteins. An important benefit of the technology is that it enables PhaseBio to use natural or modified amino acid sequences, once fused to the peptide to ELP protects against degradation by enzymes in the circulation and the fusion protein retains similar potency to the native peptide or protein. These constructs are produced in the soluble fraction of E. coli, allowing for ease of scale-up and purification.

For pharmaceutical applications as illustrated above, phase transition from a solution to a coacervate is triggered by a specific temperature shift such as to body temperature following administration, and several occurs as the concentration of the ELP fusion disrupts at the propertly of the subcutaneous injection site. Once product enters the circulation, the extended polymeric structure of ELPs provides a large hydrodynamic radius that creates a prolonged circulating half-life. This dual mechanism of slow release and long half-life provides a shallow peak to trough rates of drug levels in the circulation, which is key for maintaining therapeutic benefit hours weekly or even up to monthly dosing intervals.

Figure VI. Correction of F508del-CFTR chloride channels activity

Figure VII. Time-course of ELP-VIP corrector effect

Figure VIII. Best corrector & potentiator combinations

Figure IX. Comparative corrector effects