ELSEVIER
Journal of Cystic Fibrosis xx (2018) xxx–xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Original Article

Physiological and pharmacological characterization of the N1303K mutant CFTR

Samantha DeStefano [a,b], Maarten Gees [c], Tzyh-Chang Hwang [a,b,*]

[a] Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65211, United States
[b] Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, MO 65211, United States
[c] Galapagos NV, Mechelen, Belgium

Received 3 April 2018; revised 13 May 2018; accepted 21 May 2018
Available online xxxx

Abstract

Background: N1303K, one of the common, severe disease-causing mutations in the *CFTR* gene, causes both defective biogenesis and gating abnormalities of the CFTR protein. The goals of the present study are to quantitatively assess the gating defects associated with the N1303K mutation and its pharmacological response to CFTR modulators including potentiators VX-770 and GLPG1837 and correctors VX-809, and VX-661.
Methods: Gating behavior and pharmacological responses to CFTR potentiators were assessed using patch-clamp technique in the excised, inside-out mode. We also examined the effects of GLPG1837, VX-770, VX-809 and VX-661 on N1303K-CFTR surface expression using Western blot analysis.
Results: Like wild-type (WT) CFTR, N1303K-CFTR channels were activated by protein kinase A-dependent phosphorylation, but the open probability (P_o) of phosphorylated N1303K-CFTR was extremely low (~0.03 vs ~0.45 in WT channels). N1303K mutants showed abnormal responses to ATP analogs or mutations that disrupt ATP hydrolysis and/or dimerization of CFTR's two nucleotide-binding domains (NBDs). However, the P_o of N1303K-CFTR was dramatically increased by GLPG1837 (~17-fold) and VX-770 (~8-fold). VX-809 or VX-661 enhanced N1303K-CFTR maturation by 2–3 fold, and co-treatment with GLPG1837 or VX-770 did not show any negative drug-drug interaction.
Conclusion: N1303K has a severe gating defect, reduced ATP-dependence and aberrant response to ATP analogs. These results suggest a defective function of the NBDs in N1303K-CFTR. An improvement of channel function by GLPG1837 or VX-770 and an increase of Band C protein by VX-809 or VX-661 support a therapeutic strategy of combining CFTR potentiator and corrector for patients carrying the N1303K mutation.

Keywords: Cystic fibrosis; N1303K; Gating; GLPG1837; VX-770; VX-809; VX-661

1. Introduction

Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the gene for the Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a phosphorylation-activated but ATP-gated anion channel predominantly expressed in epithelial cells [1,2]. CFTR regulates the balance of salt and water across epithelial cells lining the exocrine glands, such as the submucosal glands in the lungs, the pancreatic ducts and the sweat glands. N1303K (asparagine-to-lysine mutation at position 1303) is a common, severe CF disease-causing mutation in the CFTR gene (Cystic Fibrosis Foundation Patient Registry 2016 Annual Data Report). As the newly resolved human CFTR structure has shown [3], N1303 is at the equivalent position, but in the opposite half of the CFTR molecule, as F508, deletion of which (F508del) constitutes the most common pathogenic mutation in CF. Like F508del, N1303K is a Class II folding defect mutation, which results in a

* Corresponding author at: 134 Research Park Drive, University of Missouri, Columbia, MO, United States.
E-mail address: hwangt@health.missouri.edu. (T.-C. Hwang).

https://doi.org/10.1016/j.jcf.2018.05.011
1569-1993© 2018 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

reduced number of N1303K channels in the cell membrane [4–6]. Both of these common, Class II folding defect mutations also show gating defects once they reach the cell membrane [7–10]. For mutations with both trafficking and gating defects, one therapeutic strategy is to use a combination of CFTR correctors (e.g., VX-809 or Lumacaftor, [11] that increase the number of channels in the cell membrane and CFTR potentiators (e.g., VX-770 or Ivacaftor, [12]) that enhance the activity or open probability (P_o) of the CFTR channel.

Although the FDA approved the use of Orkambi (Lumacaftor plus Ivacaftor) for the treatment of patients homozygous for the F508del mutation [13], the clinical benefits are somehow limited partly due to the undesirable drug-drug interaction: Chronic application of Ivacaftor dampens the effects of Lumacaftor [14,15] and the mechanisms are complex and may be mutation-specific [16–18]. While clinical trials for use of ivacaftor in combination with other first- and next-generation correctors for F508del heterozygotes bearing a minimal function mutation (e.g. N1303K) on their second allele are ongoing, in vitro results establishing effects of potentiator VX-770 on the N1303K mutation have not been published. In addition, prior to the discovery of efficacious CFTR potentiators, functional studies of CFTR mutants may have underestimated the severity of gating defects associated with these mutations (see ref. [19] for example). Thus, although Berger et al. [7] showed a P_O of ~0.1 for N1303K [7], this should be considered as a maximal value before more thorough studies are possible.

Recent development of new CFTR potentiators provides powerful tools that afford more accurate assessment of the gating abnormalities caused by CFTR mutations. For example, Yeh et al. [20] showed that the potentiator GLPG1837 is ~3-fold more efficacious than VX-770 (ivacaftor) on G551D-CFTR [20], the third most common pathogenic mutation with distinct gating defects [21]. Interestingly, the same report also provided evidence that GLPG1837 and VX-770 share a similar mechanism of action perhaps by binding to the same binding site. Despite its lower potency than VX-770, the high efficacy of GLPG1837, if also true for the N1303K mutation, should make this compound particularly valuable for a more accurate quantification of the gating defect associated with N1303K-CFTR.

In this present study, we used the CFTR potentiator GLPG1837 as a tool to estimate a maximal P_O of ~0.03 for N1303K. This gating defect likely is caused by dysfunction of CFTR's NBDs as N1303K-CFTR responds to ATP and ATP analogs very differently from WT channels. Furthermore, mutations disrupting ATP hydrolysis or NBD dimerization fail to affect N1303K channel gating. However, CFTR potentiators such as GLPG1837 and VX-770 dramatically improved the gating function of N1303K-CFTR, suggesting a clinical usefulness of these reagents. Furthermore, we showed that the surface expression of N1303K-CFTR can be enhanced by CFTR correctors VX-809 or VX-661. Therapeutic strategy of combining CFTR potentiator and corrector for patients carrying the N1303K mutation, and the potential structural mechanism for the gating defects caused by the mutation will be discussed.

2. Materials and methods

2.1. Cell culture and transfection

Chinese hamster ovary (CHO) cells were grown in 5% CO_2 at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cDNA construct of N1303K-CFTR was co-transfected with pEGFP-C3 (Clontech Laboratories, Inc.) encoding the green fluorescence protein using PolyFect transfection reagent (QIAGEN) according to the manufacturer's instructions. The transfected CHO cells were plated on sterile glass chips in 35-mm tissue culture dishes and incubated at 27 °C for 2–6 days before patch-clamp experiments.

2.2. Western blot analysis

CHO cells, in 35 mm dishes, were transfected with various DNA construct using X-tremeGENE (Roche). Six hours after transfection, drugs were added to the medium to desired concentrations. Cells were lysed 18 h post drug treatment using 1xSDS loading buffer. Cell lysates were sheared by pushed through 18G needles. Whole cell lysate were separated in 4–20% gradient gels (Bio-Rad Laboratories) and transferred onto nitrocellulose membranes. The membranes were blocked with 5% milk in TBST buffer (20 mM Tris, 137 mM NaCl, 0.1% Tween 20) at 4 °C overnight. The membranes were then probed with anti-CFTR antibody (1:3000 dilution) (AB596 from Cystic Fibrosis Foundation Therapeutics) and anti-Vimentin antibody (1,3000 dilution) (Santa Cruz Biotechnology) at room temperature for 2 h. The membranes were washed with TBST five times and then incubated with anti-mouse IgG, HRP linked antibody (Cell Signaling Technology) at room temperature for 1 h. The membranes were washed three times with TBST and developed with chemiluminescence reagent (Thermo Scientific). The luminescence was detected by a Molecular Image Chemi Doc (Bio-Rad Laboratories).

2.3. Electrophysiological experiments

Details of patch-clamp experiments were described in our previous publication [22]. The pipette solution contained (in mM): 140 NMDG chloride (NMDG-Cl), 2 $MgCl_2$, 5 $CaCl_2$, and 10 HEPES, pH 7.4 with NMDG. Cells were perfused with a bath solution containing (in mM): 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 5 HEPES, and 20 sucrose, pH 7.4 with NaOH. After the establishment of an inside-out configuration, the patch was perfused with a solution containing (in mM): 150 NMDG-Cl, 2 $MgCl_2$, 10 EGTA, and 8 Tris, pH 7.4 with NMDG.

CFTR channel currents in inside-out patches were recorded at room temperature with an EPC-10 patch clamp amplifier, filtered at 100 Hz with an eight-pole Bessel filter (Warner Instrument Corp.) and captured onto a hard disk at a sampling frequency of 500 Hz. The membrane potential was held at −30 or − 50 mV and the inward current was inverted for clear data presentation. CFTR was first activated by cytoplasmic

Please cite this article as: DeStefano S, et al, Physiological and pharmacological characterization of the N1303K mutant CFTR..., J Cyst Fibros (2018), https://doi.org/10.1016/j.jcf.2018.05.011