Published in final edited form as:

Curr Opin Pharmacol. 2017 June ; 34: 98–104. doi:10.1016/j.coph.2017.09.015.

CFTR potentiators: From Bench to Bedside

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Abstract

One major breakthrough in cystic fibrosis research in the past decade is the development of drugs that target the root cause of the disease—dysfunctional CFTR protein. One of the compounds, Ivacaftor or Kalydeco, which has been approved for clinical use since 2012, acts by promoting the gating function of CFTR. Our recent studies have led to a gating model that features an energetic coupling between NBD dimerization and gate opening/closing in CFTR's TMDs. Based on this model, we showed that ATP analogs can enhance CFTR gating by facilitating NBD dimerization, whereas Ivacaftor works by stabilizing the open channel conformation of the TMDs. This latter idea also explains the near omnipotence of Ivacaftor. Furthermore, this model marks multiple approaches to synergistically boost the open probability of CFTR by influencing distinct molecular events that control gating conformational changes.

Introduction

Cystic fibrosis (CF), a fatal genetic disease afflicting mainly people of Caucasian origin, is caused by malfunction of Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a chloride ion channel involved in transepithelial salt and water movement [1,2]. While pathogenic mutations in CFTR could result in a variety of biochemical/biophysical defects to perturb the secretory or absorptive function of epithelial cells [3,4], many of these disease-associated mutations, including the most prevalent one F508-CFTR, cause abnormal gating (i.e., a reduced open probability, P_o). Thus, searching for reagents (CFTR potentiators) to restore the channel function of CFTR has been of great interest as it may lead to new treatment options of CF for patients carrying such mutations.

Just like other members of the ABC (ATP-Binding Cassette) Transporter Superfamily, the CFTR protein is made of five distinctive domains, namely two transmembrane domains (TMDs), two nucleotide-binding domains (NBDs) and a regulatory (R) domain. It is well

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established that CFTR's R domain needs to be phosphorylated by protein kinase A (PKA) before the channel can be gated by ATP [5]. While the molecular mechanism for ATP-dependent gating remains to be elucidated [6,7], it is generally accepted that gate opening is coupled to ATP binding-induced dimerization of CFTR's two NBDs in a head-to-tail configuration and gate closure is promoted by ATP hydrolysis at the catalysis-competent site (or site 2 composed of the head subdomain of NBD2 and the tail subdomain of NBD1) [8]. Although the detailed mechanism by which CFTR's NBDs are coupled to the gate in the TMDs remains debated [9]. For the purpose of discussion, in the current article we choose to use a model that conveniently explains mutation-induced gating defects as well as the action of CFTR potentiators (Figure 1). For example, the third most common disease-associated mutation G551D, also the first mutation subjected to targeted therapy (see below), has been shown to exhibit an extremely low P_o (less than 1% of the P_o of wild-type channels) likely due to a defect in ATP-induced NBD dimerization [10–12]. Thus, the rightmost horizontal transitions in Figure 1 are abolished by the G551D mutation. What is left for G551D-CFTR is ATP-independent spontaneous openings (see details in ref [13]).

In this review, we will briefly review the history of studies on reagents that work as CFTR potentiators, discuss possible mechanisms of action for different agents, and elaborate ongoing challenges that need to be overcome to realize personalized medicine for cystic fibrosis.

ATP analogs and small-molecule CFTR potentiators: proof of concept

Since NBD dimerization plays a critical role in gate opening, a potentiator designed to promote this process could in theory be of great use. One intuitive thought is to modify CFTR's natural ligand ATP to create analogs with higher affinity and efficacy in catalyzing NBD dimerization. Targeting CFTR's two ATP-binding sites indeed works! For example, addition of a small functional group to the N⁶ position of the adenine ring has been demonstrated to significantly increase the P_o of CFTR. Among the three ATP analogs studied by Zhou and colleagues [14], N⁶-(2-phenylethyl)-ATP (P-ATP) is the one with the highest apparent affinity and efficacy. Indeed, P-ATP has been shown to increase the P_o of G551D-CFTR (Figure 2) and F508-CFTR by 7- and 5- fold respectively [15]. The main effect of P-ATP on G551D-CFTR is through an unexpected action on the catalysis-incompetent site (or site 1 composed of the head subdomain of NBD1 and the tail subdomain of NBD2). A different ATP analog 2'-deoxy-ATP (2'-dATP) also enhances gating of wild-type (WT) [12,16] and G551D-CFTR [12]. Remarkably, N⁶-(2-phenylethyl)-2'-deoxy-ATP (P-dATP), a structural combination of P-ATP and dATP, increases the P_o of G551D-CFTR by 36-fold as if these two chemical modifications in ATP act synergistically in potentiating G551D-CFTR gating [15]. Of note, accomplishing this level of opening probability for G551D-CFTR *in vivo* is expected to provide a functional cure for patients carrying such mutation. Although P-dATP also completely rectifies the gating defect in F508-CFTR [15], its clinical utility is limited as ATP analogs are unlikely to become therapeutically useful due to bioavailability issues as well as potential non-specific interaction with countless ATP binding proteins involved in different physiological functions. Nonetheless, these studies using ATP analogs do provide proof-of-concept

evidence for the feasibility of correcting gating defects for at least some of the most common pathogenic mutations.

The desire to find small-molecule CFTR-specific potentiators presents itself as a long list of chemicals that improve CFTR gating reported even a decade before the dawn of success in developing CFTR-targeted drugs [17]. As the cAMP-PKA signaling pathway constitutes the physiologically key step for CFTR activation, the first compound tested is IBMX (3-isobutyl-1-methylxanthine). Drumm *et al.* [18] proposed that IBMX, by inhibiting phosphodiesterase, increases intracellular cAMP and consequently PKA activity so that

F508-CFTR can be activated to a higher level. Then, Illek *et al.* [19,20] demonstrated that genistein, a tyrosine kinase inhibitor found in many foods, can activate CFTR in intact cells through a mechanism indistinguishable from that of forskolin, an adenylyl cyclase activator. Three later studies however independently demonstrated that the CFTR protein itself is the target for genistein, establishing for the first time that CFTR itself can serve as a target for small-molecule CFTR potentiators [21–23]. Besides genistein, other flavonoids molecules have also been shown to enhance CFTR function both *in vitro* and *in vivo* [24].

Another natural compound curcumin has also drawn significant interests as a potential therapeutic agent for CF. Curcumin was first reported as an effective CFTR corrector; it improves maturation of F508-CFTR ([25]; but cf. [26,27]). Similar to genistein, however, curcumin increases the P_o of WT-CFTR by extending the opening burst duration and decreasing the interburst duration [28]. Moreover, curcumin potentiates F508-CFTR to a similar extent as that of genistein [28]. Also like genistein [29], curcumin works as a CFTR potentiator for G551D-CFTR [30]. Furthermore, the observation that curcumin enhances the activity of NBD2-CFTR, a mutant with entire NBD2 deleted, indicates that curcumin acts through a mechanism that does not require ATP binding or NBD dimerization [30].

Evidence regarding the additive effect of genistein and curcumin for gating potentiation has been controversial. Berger and colleagues showed that in excised inside-out patches, genistein inhibits curcumin-stimulated WT-CFTR current in a dose dependent manner [28] whereas a later study by Yu *et al.* reported the opposite [31]. In both whole cell and cell-attached patches containing G551D-CFTR, genistein and curcumin demonstrated an additive effect with a maximum 40-fold increase of CFTR currents under saturating concentrations [31,32]. This apparent controversy might be due to different experimental conditions and/or contributions of intracellular factors.

VX-770 (Ivacaftor): a milestone in personalized medicine for CF

In 2009, by using high throughput drug screening, Vertex Pharmaceuticals identified a compound, VX-770, that potently increases the activity of F508- and G551D-CFTR in excised ‘cell-free’ membrane patches (Figure 3), and significantly augments Cl^- secretion in cultured CF bronchial epithelia isolated from patients carrying these mutations [33]. In subsequent phase II and phase III clinical trials [34,35], VX-770 effectively improved predicted FEV1 (Forced Expiratory Volume in One second), decreased sweat chloride concentration and reduced the frequency of pulmonary exacerbations, all indicative of a beneficial clinical outcome. While the original clinical trials involved only patients carrying

the G551D mutation, VX-770 (Ivacaftor or Kalydeco) was eventually approved for a variety of mutations that cause gating dysfunction [36–38]. The approval of VX-770 by the FDA in 2012 ushered in a new era of precision medicine that targets directly the underlying cause of CF.

The invention of Ivacaftor was no doubt one of the most important breakthroughs in the search for an ultimate cure of CF. However, very little was known about its mechanism of action at the time of its launch. It was reported [33,39,40] that VX-770 increases the P_o of WT-CFTR by boosting its opening burst duration, which was thought to be dependent on the rate of ATP hydrolysis in CFTR's site 2 [6,41]. However, it is unlikely that VX-770 acts through delaying ATP hydrolysis because it is effective in hydrolysis-deficient mutants such as G551D [33,39,40] and E1371S [40], as well as in WT-CFTR in the complete absence of ATP [39,40].

Although more studies are needed to eventually illuminate how VX-770 works at a molecular level, the gating model that features an energetic coupling between NBD dimerization and gate opening/closing in the TMDs depicted in Figure 1 suffices to explain nearly all the experimental data with VX-770. We proposed that in the continuous presence of ATP, VX-770, by binding to WT-CFTR's TMDs, shifts the gating transitions towards the open state in the three vertical steps in Figure 1 ($C_2AD \leftrightarrow O_1$, $C_2ATP \leftrightarrow O_2ATP$, $C_2 \leftrightarrow C_2$) [39]. Thus, regardless of whether the ATP-induced NBD dimerization is intact as in the case of WT channels, or defective as in G551D-CFTR, whether ATP is hydrolyzed or not (for E1371S), and even whether the channel possesses two NBDs or not (in NBD2-CFTR), VX-770 will improve gating function [13,39,40,42].

The basic research conducted *in vitro* provides the mechanistic basis for the near omnipotence of VX-770 in promoting the gating function of CFTR mutants. However, reagents other than VX-770 will be needed to rectify biochemical defects that are insensitive to VX-770. Furthermore, for reasons described below, there is an urgent need to develop new CFTR potentiators that may complement or supplant VX-770. To these ends, several recent biotechnological innovations provide tools and platforms for drug testing tailored to a specific genetic background often to the level of an individual patient. For example, rectal organoids derived from tissues collected in CF patients offer a highly precise and sensitive approach to test CFTR function and its pharmacological response based on an individual's genetic background [43]. Furthermore, the cutting-edge CRISPR technique has been successfully employed to create F508 genotype in specific cell lines [44]. These new inventions add powerful arsenals to the already rich resources for the development of novel therapy in CF.

Ongoing challenges and future perspectives

VX-770 is no doubt a remarkable CFTR potentiator. However, for the most prevalent mutant F508-CFTR, clinical trials with Ivacaftor alone failed to attain satisfying outcomes [45] probably due to multiple defects associated with the F508 mutation. Interestingly, even with a combination regiment of VX-770 and VX-809 (aka Lumacaftor, a CFTR corrector, [46]), only moderate improvement was observed in clinical trials [47,48]. Two recent papers

present *in vitro* experimental data supporting the notion that a prolonged exposure of F508-CFTR channels to VX-770 negatively impacts the effect of a CFTR corrector, VX-809, on CFTR trafficking and stability [49,50]. Therefore, finding second-generation CFTR potentiators without this undesirable drug-drug interaction is an imminent task.

It should be noted that the negative impact of VX-770 on VX-809 observed in F508-CFTR could be mutation specific. It is possible that this combination regimen can benefit patients carrying mutations other than F508 since these two drugs act on two completely different steps for CFTR function. Lately, it was also shown that chronic co-treatment with Lumacaftor plus Ivacaftor restores stability of a small sub-population of F508-CFTR Cl⁻ channels with a majority of the mutants remaining destabilized [51]. A recent study showed that VX-770 acts additively with VX-809 to restore CFTR function in chronically treated R117H/ F508 cells [52]. Surprisingly however, increased chloride conductance was not seen in W1282X cells despite the presence of adequate transcript levels when treated with both VX-770 and VX-809 [53]. As the combination of VX-770 and VX-809 (i.e., Orkambi) is now used clinically for treating patients carrying the F508 mutation, it seems fitting to rigorously examine its effects on other CF mutations to potentially extend its application.

As described above, studies of CFTR gating modulation by ATP analogs and small-molecule experimental reagents have established pharmacological synergism in promoting CFTR gating for WT as well as mutant CFTR. One potentially fruitful approach is to identify chemicals that work synergistically with VX-770. Recent reports indeed verified this possibility at least for G551D and R117H [54,55]. These studies also provide evidence for multiple drug binding sites that may accommodate reagents with vastly different structures. The notion that these binding sites reside in different domains of CFTR also raises the possibility of structure-based drug design especially in light of the latest breakthrough in solving the first atomic structure of the whole CFTR protein [56].

Conclusions

Over the past two decades, significant accomplishments have been made in CFTR pharmacology. The success in developing VX-770 (Ivacaftor) is an important milestone in our unwavering commitment to bringing personalized medicine to fruition for patients with CF. It also serves as a template for designing next-generation targeted therapy for CF. Nonetheless, continuous efforts from different fields and different scientific disciplines are needed in our pursuit of an ultimate cure for all CF patients. To our delight, recent advancements in all aspects of CF research have laid a solid foundation for an optimistic outlook that tailored therapy for each CF patient is within our reach.

Acknowledgments

We thank all our colleagues for their constant encouragement and stimulating discussion. The work is supported by NIH R01-DK55835 and a grant from the Cystic Fibrosis Foundation (Hwang11P0).

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Highlight

Mechanistically different CFTR potentiators synergistically promote CFTR gating

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Figure 1. A gating scheme for WT-CFTR

An energetic coupling model modified from [7]. The top four states represent the closed states, whereas the lower four states are open channel conformations. This scheme illustrates the relationship between opening/closing of the gate in the TMDs (four vertical transitions), ATP binding/unbinding (two equal length horizontal transitions in the middle), NBD dimerization/dissociation (the leftmost and rightmost horizontal transitions and AD means after dimerization with NBDs occupied by ATP), and ATP hydrolysis and dissociation of hydrolytic products (depicted as an unidirectional arrow). The yellow trapezoids represent CFTR's two TMDs, which craft a gated pore. The red and blue semicircles depict CFTR's NBD1 and NBD2 respectively. They jointly form the catalysis-incompetent site 1 (lower binding pocket), and catalysis-competent site 2 (upper binding pocket). The different lengths of the arrows marking the transitions represent relatively different rate constants. The red arrows mark the two defective transitions in G551D, and blue arrows represent those affected by VX-770. Of note, even for the closed states, TMD conformations will change along with NBD dimerization. We propose that the recently solved closed channel structure with dimerized NBDs [57] represents the C_2AD state in the scheme. A latest report also suggests that the O_2 state may assume a different pore structure than the O_1 state. Specifically, the internal vestibule of CFTR's pore is wider in the O_2 state than the O_1 state [58].

Figure 2. Hydrolyzable ATP analogs as gating potentiators

A. The chemical structure of P-ATP, dATP and P-dATP. B. A representative trace of G551D-CFTR treated with 50 μ M P-dATP. The current increased around 35-fold after switching the ligand from 1 mM ATP to 50 μ M P-dATP. (Reprinted from The Journal of Biological Chemistry 285, 19967-19975 with permission from the authors).

Figure 3. Effects of VX-770 on G551D-CFTR

Representative single-channel current traces of G551D-CFTR in the absence or presence of 200 nM of VX-770. Of note, in the presence of VX-770, there are more opening events in the same duration of recording (i.e., an increased opening rate), and the opening bursts are significantly prolonged compared to those in the absence of VX-770 (i.e., a decrease of the closing rate). These two kinetic effects of VX-770 seen in all CFTR constructs we have tested [13, 40, 42, 54, 55, 59], increase the P_o of G551D-CFTR by ~ 10 -fold so that the presence of the second channel is revealed, highlighting the technical difficulty in estimating the number of function channel in a patch-clamp experiment for mutants such as G551D with an extremely low P_o (see ref [10] for details).