PHARMACOLOGICAL THERAPY OF CYSTIC FIBROSIS BASIC DEFECT

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BACKGROUND

Cystic fibrosis (CF) is characterized by abnormal ion transport in various epithelia (1, 2). Mutations in CF patients cause loss of function of CFTR, a plasma membrane protein involved in cAMP-dependent Cl^- secretion. CFTR defect causes obstruction of airway submucosal glands and pancreatic ducts. It is believed that CFTR has also the ability to reduce, through a regulatory mechanism, the extent of Na^+ absorption occurring through the ENaC channel (3). In the surface epithelium of the airways, combination of defective Cl^- secretion and excessive Na^+ absorption causes a reduction of the periciliary fluid (PCF) volume with a consequent collapse of the mucous layer entrapping the cilia (4). The consequent impairment of the mucociliary clearance would favor the bacterial colonization of the airways. It is also possible that bacterial persistence in the CF airways is caused by the obstruction of submucosal glands which cannot secrete proteins with antimicrobial activity (5).

The CFTR protein belongs to the superfamily of ABC (ATP-binding cassette) proteins (6, 7). In particular, CFTR is part of ABCC subfamily which also includes multidrug resistance proteins (MRP) and sulphonylurea receptors (SUR). ABC protein structure consists in transmembrane helices (12 in CFTR) and two nucleotide binding domains (NBD1 and NBD2) that are exposed to the cytosol (7). The NBDs have the ability to bind and hydrolyze ATP. This process is needed to fuel the active transport of various molecules by most of ABC proteins. In contrast to all other members of its family, CFTR is not an active transporter but rather an ion channel. Indeed, interaction of ATP with NBDs is utilized to gate a transmembrane pore that is permeable to Cl^- and other anions including HCO_3^-.

In the last years various *in vitro* studies have demonstrated the possibility of pharmacological intervention to correct the primary defect in CF (8 – 10). This may be obtained by directly addressing the CFTR protein or by modulating the activity of other types of ion transport in epithelial cells like Ca^{2+}-dependent Cl^- channels.

CLASSES OF CF MUTATIONS

The development of pharmacological approaches aiming at restoring the activity of CFTR protein in CF patients needs to take into account the different mechanisms through CF mutations cause loss of function. CF mutations have been grouped in five classes (11). Class I consists in mutations that introduce a premature stop codon in the CFTR coding sequence. Class II includes deltaF508 and is characterized by mutations that cause a maturation defect in CFTR protein (12 – 14). The mutant protein is retained in the endoplasmic reticulum and degraded in the proteasome. Class III and IV mutations do not impair the biogenesis of CFTR protein but decrease its ability to transport Cl^-. In class III mutants (e.g. G551D and G1349D), this is due to impairment of NBD function and channel gating (15, 16). Consequently the CFTR channel remains for most of the time in the closed state. Conversely, class IV mutations (e.g. R347P and R117H) affect the CFTR channel pore reducing its permeability to Cl^- (17). Class IV mutations are tipically mild, causing only a partial loss of function and therefore a non severe clinical phenotype. Finally, class V is characterized by a decrease in the synthesis of CFTR protein (18 – 20). The mechanism is mainly due to alteration of the mRNA splicing process causing the synthesis of an abnormal non functional protein. The splicing defect is generally partial. Therefore, there is production of normal CFTR protein whose level of expression is variable within individuals and among different tissues.

It is important to point out that deltaF508 is not a pure class II mutation. If the deltaF508 mutant protein is allowed to reach the plasma membrane by incubating the cells at low temperature, it shows also a gating defect which is however less severe than that of typical class III mutations (21).

PHARMACOLOGICAL CORRECTION OF CFTR MUTATIONS

Table I reports the various compounds that have been found to restore activity of mutant CFTR in vitro, and for a few of them, also in vivo on CF patients. Some of the reported compounds (e.g. genistein) have been used extensively as a tool of research to understand the mechanism of CFTR gating. Hopefully, the newly discovered correctors of ΔF508 will be also useful to identify the steps involved in CFTR protein biogenesis and ΔF508 mistrafficking. Development of effective drugs for CF patients will need further validation and optimization of the most promising active compounds. This process is seriously limited by the lack of an useful animal model. Indeed, ΔF508 mice do not display a clear pathology resembling the lung disease of CF patients. Therefore, it will be very important to develop novel assays to evaluate candidate drugs for CFTR pharmacotherapy. These assays may be based on relevant end points like ASL properties, mucociliary transport, and sumucosal gland secretion.

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Table 1 – Pharmacological correction of CFTR mutations

CLASS I MUTATIONS (e.g. G542X, W1282X)		
Compound	**Studies**	Comments
Aminoglycoside antibiotics (gentamicin, tobramycin, geneticin)	Correction of stop codon mutations. In vitro and in vivo studies (22 – 25).	These compounds work by inducing the ribosome to read through the stop codon. An aminoacid is inserted at the level of the stop codon allowing the continuation of CFTR protein synthesis. The insertion may not be random. Some amino acids may be preferred over others. Supporters of this approach report no major cell toxicity by aminoglycosides at the concentrations that suppress stop codons. It is possible that premature stop codons are more specifically affected than normal stop codons present in all genes. Two studies demonstrated significant correction of Cl^- secretion in CF patients by nasal potential measurements (24, 25). A company (PTC Therapeutics) is testing a new compound, PTC124, as a suppressor of stop codon mutations in CF and other genetics diseases.
CLASS II MUTATIONS (ΔF508): CORRECTORS		
Compound	**Studies**	**Comments**
4-phenylbutyrate	Correction of ΔF508 in vitro and in vivo (26 – 29).	Effective at millimolar concentrations. Possible mechanism based on changes in expression of intracellular chaperones (27). Small clinical trials showed significant correction of Cl^- secretion after systemic administration (28, 29).
curcumin thapsigargin	Correction of ΔF508. In vitro and animal studies (30, 31).	Correction at the biochemical and functional level. Improved survival of ΔF508 mice. Putative mechanism: alteration of Ca^{2+} homeostasis in the endoplasmic reticulum with changes in chaperone function. Activity of curcumin and thapsigargin questioned by other investigators which found no correction on the ΔF508-CFTR protein and in ΔF508 mice (32 – 35).

MPB-07 MPB-91	Correction of ΔF508. In vitro and ex vivo study (36).	Compounds previously identified as CFTR activators. Correction at the functional level (iodide efflux) after incubation of ΔF508 cells. Improved targeting of the mutant protein to the plasma membrane revealed by immunofluorescence.
bisaminomethyl-bithiazole (corrector-4a)	Identification by high-throughput screening (37). In vitro study.	Correction at the functional (yellow fluorescent protein assay, short-circuit current recordings) and biochemical level (immunodetection). Activity at low micromolar concentrations. Mechanism based on improved folding efficiency and improved stability at the plasma membrane. Activity confirmed in CF bronchial epithelial cells. Partial correction.
quinazolines VRT-325 VRT-422	Identification by high-throughput screening. In vitro studies (38, 39).	Identified at Vertex Pharmaceuticals. Correction demonstrated at the functional and biochemical levels. Activity at low micromolar concentration. Mechanism based on improved exit of mutant protein from endoplasmic reticulum. Activity confirmed in CF bronchial epithelial cells. Partial correction.
S-nitrosylating agents GSNO	Effect on wild type and ΔF508 CFTR. In vitro studies (40, 41).	Increase of immature and mature form of ΔF508 protein upon exposure to GSNO and other agents. Increase of Cl^- transport. Possible mechanisms: transcriptional effect on CFTR expression plus upregulation of cysteine string proteins (Csp1 and 2).

CLASS III MUTATIONS AND ΔF508: POTENTIATORS		
Compound	*Studies*	*Comments*
genistein apigenin benzoflavones	Electrophysiological analysis of compound activity on normal and mutant CFTR. In vitro and in vivo studies (42 – 47).	Flavonoids activate wild type and mutant CFTR (including ΔF508 and G551D). Rescue of G551D activity requires high micromolar concentration. At very high concentrations, genistein causes CFTR inhibition. Active benzoflavones were found by screening a small library of apigenin and genistein analogs (47).
xanthines (IBMX, CPX, X-33)	Activation of wild type and mutant CFTR. In vitro studies (48, 49).	Xanthines include phosphodiesterase inhibitors and therefore may activate wild type CFTR by increasing cAMP. However, some xanthines, including IBMX and X-33, are probably direct CFTR activators and rescue the channel gating defect of mutants. IBMX increases the open channel probability of ΔF508-CFTR. CPX is poorly effective on the G551D mutant (46).
MPB-07 MPB-91 MPB-104	Activation of wild type and mutant CFTR (50 – 52).	The MPB-91 and MPB-104 benzo[c]quinolizinium compounds are effective activators of the G551D mutant.
benzimidazolones (NS004)	Electrophysiological analysis of compound activity on normal and mutant CFTR (53, 54).	NS004 is a potent activator of the ΔF508 mutant. Mechanism of activation in common with genistein.
tetrahydrobenzo-thiophenes	Identification by high-throughput screening (100,000 compounds) (55).	Compounds active at nanomolar concentrations on the ΔF508 mutant. The same screening, performed on 100,000 compounds, detected other five classes of active compounds.
phenylglycines sulfonamides	Identification by high-throughput screening (50,000 compounds) (56).	Compounds active at nanomolar concentrations on the ΔF508 mutant. Phenylglycines are also strongly active on the G551D and G1349D mutants. Mechanism based on increased open channel probability of CFTR mutants.
capsaicin	Electrophysiological analysis of compound activity (57).	Effective on ΔF508 and G551D.

fluorescein derivatives (phloxine B)	Electrophysiological analysis of compound activity (58, 59).	Phloxine B is active on the ΔF508 mutant. Dose-response relationship is bell-shaped, with high concentrations causing CFTR inhibition. Direct effect on CFTR channel.
phenantrolines benzoquinolines	Analysis of Cl^- transport in murine tissues (60).	Activation of CFTR-dependent Cl^- secretion. Effect mediated also by activation of basolateral K^+ channels.
VRT-532	Identification by high-throughput screening (38).	Found at Vertex Pharmaceuticals. Compound active at micromolar concentrations. Activity on the ΔF508 mutant confirmed in CF bronchial epithelial cells.
felodipine	Identification by screening a 2,000 compound drug library (61).	Antihypertensive 1,4-dihydropyridines, including felodipine, nimodipine, and nicardipine, activate ΔF508 and G551D CFTR. The mechanism is not due to block of Ca^{2+} channels but probably to direct interaction with CFTR protein.