Rescuing ΔF508 CFTR with trimethylangelicin, a dual-acting corrector and potentiator

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Cystic Fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CF gene (31). The gene product encodes a protein called the cystic fibrosis transmembrane conductance regulator (CFTR), a 1,480-amino acid protein that is a member of the ATP-binding cassette (ABC) transporter superfamily (30). CFTR is a Ca²⁺-activated anion channel and is composed of two nucleotide-binding domains (NBD1 and NBD2), two membrane-spanning domains (MSD1 and MSD2), a regulator region (R), and four cytosolic loops (CL1–4) (29). More than 1,900 mutations have been identified in the CFTR gene (http://www.genet.sickkids.on.ca/cftr/StatisticsPage.html). ΔF508 CFTR is the most common mutation and this mutation results in the production of a misfolded protein that is degraded by the ubiquitin-proteasome system during biogenesis (2, 39).

Understanding how loss of phenylalanine at position 508 in NBD1 affects the folding of CFTR has turned out to be a complex problem. Although ΔF508 CFTR is subject to ubiquitin-dependent endoplasmic reticulum (ER)-associated degradation during biogenesis, low-temperature incubation (27°C) of cultured cells for 24–48 h facilitates delivery of some maturely glycosylated ΔF508 CFTR to the cell surface (7). And although this maturely glycosylated protein manages to escape degradation, there still appear to be some defects associated with its channel activity and plasma membrane surface stability (3, 5, 12, 13, 18, 33, 38). Despite these additional concerns, there has been considerable effort in the CF field to identify small molecule “correctors” that promote the correct folding and exit of ΔF508 CFTR from the ER and delivery to the plasma membrane (24, 37, 40).

Because of multidomain structure of CFTR, it has been proposed that the sequential folding of CFTR requires the stabilization of both NBD1 energetics and NBD1 interactions between MSD1 and MSD2 (10, 11, 20, 23, 27, 28, 35). This has led to the concept that the different correctors that have been identified can only fix one of the folding defects of ΔF508 CFTR but not others, and therefore these correctors fall into distinct classes or complementation groups (20, 23). This further supports the idea that a combination of drugs is required to fix ΔF508 CFTR. The most promising corrector to date, Vertex 809, increases the functional activity of ΔF508 CFTR in vitro to ~15% of wild-type CFTR in primary human bronchial epithelial cells, and the addition of a “potentiator” such as Vertex 770 that increases channel gating properties of ΔF508 CFTR has additive functional effects (36). Despite these exciting advances, it is unclear whether additional therapeutic approaches will be necessary to achieve the needed therapeutic benefit for patients.

Most approaches have considered combinations of a corrector and a potentiator as described above for the Vertex compounds or, alternatively, combinations of different classes of correctors. The latter strategy has certainly been shown to have additive effects (16). An alternative approach has been to identify single compounds with both corrector and potentiator effects, which has proven to be more difficult, although examples of these have been found (14, 25, 26). One clever approach was to construct a hybrid potentiator-corrector molecule using two known compounds with these activities that would subsequently be cleaved in the intestine into its two bioactive components (21). Another approach has been to identify correctors that also test positive for potentiator activity; CoPo-22 is an example of such a compound (26, 32).

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Clearly to date, no one compound is available that appears to be sufficient for demonstrating a clinical benefit in patients expressing ΔF508 CFTR, and therefore the search for drug combinations that push through this apparent therapeutic glass ceiling continues.

A recent paper by Favia et al. (9) continues with this idea of dual-acting compounds, but in this case that is not what the authors were originally searching to find. The initial studies were directed toward identifying compounds that inhibited IL-8 production and the subsequent recruitment of neutrophils into the bronchi of CF patients (22). Given that neutrophils promote lung inflammation and activate sodium channels (1), blocking this response is a key therapeutic target in CF (15). In the initial studies using plant extracts, and then later using purified components from active extracts, the authors identified 5-methoxypsoralen as a compound that inhibited IL-8 expression at a 10 μM concentration in bronchial epithelial cells when these cells were challenged with Pseudomonas aeruginosa (22). In their follow-up studies examining analogs of 5-methoxypsoralen, Tamanini et al. (34) found that the most potent analog, 4,6,4-=trimethylangelicin (TMA), inhibited P. aeruginosa-dependent IL-8 in the nanomolar concentration range in a number of different human airway epithelial cell lines. Furthermore, because psoralens were known to potentiate CFTR-mediated channel function (8), the authors tested TMA and found that it had potentiator activity (34).

In their most recent study, Favia et al. (9) tested whether TMA can act as a corrector as well, and, if so, how it compares to Vertex 809. Using Fischer rat thyroid (FRT) cells and a fluorescent YFP halide sensor, the authors demonstrated that 48-h treatment with TMA enhanced iodide influx as monitored by fluorescence quenching and found that the maximum activity occurred at a 200 nM concentration. The specific CFTR inhibitor, CFTRinh-172 (19), was used to validate the specificity of this response. Interestingly, the magnitude of the response was greater in the FRT cells with the TMA (200 nM) than with Vertex 809 (10 μM) in these assays. The TMA effect was also shown in polarized CFBE41o-ΔF cells, and, after 24 h of treatment with TMA, the CFTR-dependent chloride efflux was stable for an additional 24 h after drug washout, indicating that the TMA effectiveness appears to be long lasting (9). This suggested that TMA had two important properties of a corrector: it corrected the biogenesis defect and it stabilized ΔF508 CFTR at the cell surface even after drug washout (Fig. 1).

Another important aspect of the corrector treatment is finding compounds that are additive with Vertex 809. The combination therapy with 100 nM TMA and 5 μM Vertex 809 indicated that there was no additive effect, suggesting that both are in the same class of drugs and were acting via a similar mechanism (9). Furthermore, in an effort to directly compare the potentiator activity of TMA to Vertex 770 in polarized CFBE41o-ΔF cells, Favia and colleagues tested acute addition

![Fig. 1. Trimethylangelicin (TMA) effects on ΔF508 CFTR biogenesis, channel function, and plasma membrane stability. A: ΔF508 CFTR biogenesis defect. TMA promotes ΔF508 CFTR folding and exit from the endoplasmic reticulum (ER) and delivery to the plasma membrane (PM) (corrector effect). B: ΔF508 CFTR channel gating defect. TMA activates the channel at the plasma membrane (potentiator effect). C: ΔF508 CFTR is rapidly endocytosed. TMA inhibits rapid endocytosis (corrector effect). D: surface ΔF508 CFTR fails to recycle efficiently and is rapidly degraded at 37°C. TMA presumably promotes efficient recycling (corrector effect) given that the corrected protein is stable at the cell surface for more than 8 h at 37°C. WT, wild-type CFTR.](http://ajplung.physiology.org/doi/abs/10.1152/ajplung.00177.2014?journalGroup=ajplung)
of 10 μM Vertex 770 vs. 250 nM TMA after a 24-h treatment with 100 nM TMA (9). The results demonstrated that the two acute treatments were comparable (9), further suggesting that TMA effectiveness as both a corrector and potentiator (Fig. 1) were very impressive.

To understand how TMA was working, Favia and colleagues (9) found that TMA treatment had no effect on CFTR mRNA message levels in CFBE41o-ΔF cells but did enhance the levels of both immaturely and maturely glycosylated ΔF508 CFTR protein in a manner similar to Vertex 809. Low-temperature-corrected CFBE41o-ΔF cells that were pre-treated with TMA during the last 3 h of low temperature also demonstrated that the TMA effect stabilized the surface pool compared with the vehicle control (Fig. 1), suggesting that TMA treatment was effective even after biogenesis. But the in vitro acid test for a CFTR corrector has always been in testing its effectiveness in polarized monolayers of primary human bronchial epithelial cells derived from ΔF508 CFTR homozygous patients. In these experiments, the wild-type control bronchial epithelial cells were from four healthy donors, and the experimental cells were bronchial epithelial cells derived from four CF donors treated for 24 h with vehicle control or 100 nM TMA (9). The chloride efflux measurements indicated that the CFTR-dependent secretion was almost 40% of the wild-type activity, suggesting that TMA was very effective in primary cells (9). Using an alternative functional test, Favia and colleagues found that Ussing chamber analyses of primary cells (9). Using an alternative functional test, Favia and colleagues found that Ussing chamber analyses of primary cells supported the fluorescence assay results (9).

Finding a corrector and potentiator within one molecule certainly is an appealing concept. But whether this compound or an analog is sufficient to break through the therapeutic glass ceiling remains to be established. Understanding TMA’s mechanism of action is certainly the next step. Furthermore, determining how well this compound works in the primary CF intestinal organoids (6) and in animal models is important as well. Is it possible that a single drug can be used to reverse the lung pathology found in CF patients? Is a single drug a better strategy for patient care than two or more drugs? Could other therapies such as regulation of sodium channels (4, 17) or maturation without thermally stabilizing the mutant protein. To understand how TMA was working, Favia and colleagues found that Ussing chamber analyses of primary cells (9). Using an alternative functional test, Favia and colleagues found that Ussing chamber analyses of primary cells supported the fluorescence assay results (9).

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