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COMMENTARY

Designer pharmacotherapy for the treatment of cystic fibrosis: commentary on Zegarra-Moran *et al*

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Cystic fibrosis (CF) is a chronic, progressive and generally fatal genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes an epithelial anion channel. CFTR belongs to a large family of ATP-powered pumps that transport a diverse set of solutes across lipid bilayers. All members have a conserved nucleotide binding domain (NBD) that contains three highly conserved motifs required for nucleotide binding and hydrolysis; the Walker A and B sites, and the LSGGQ signature sequence. These transporters also have a common domain organization of two cytoplasmic NBDs, associated with two transmembrane domains (TMDs). Uniquely, CFTR has an additional regulatory (R) domain containing a number of conserved consensus phosphorylation sites for PKA and PKC. In this way CFTR functions as an ATP-gated anion channel regulated by PKA/PKC phosphorylation, and not a pump (as far as we know). How ATP binding/hydrolysis and phosphorylation are coupled to the opening and closing (gating) of the CFTR pore remains uncertain.

Among other effects, CF patients have impaired CFTR-mediated Cl^- and HCO_3^- secretion and enhanced amiloride-sensitive Na^+ transport across the small airways. This primary defect in ion transport depletes airways surface liquid (ASL), which leads to impaired mucociliary clearance, reduced mucous hydration and mucus impaction on airway surfaces. These factors predispose the lungs to repeated bacterial infections and tissue destruction, and most CF patients die from respiratory failure. Thus it is predicted that any improvement in ASL volume should be beneficial to the CF lung (Tarran *et al*., 2001). Although the simplest way to 'correct' this recessive disease is gene therapy, this approach is still some way from being successful and no clear clinical benefit has so far been demonstrated. Thus, finding alternate or complementary strategies remains an important goal. The last 3–5 years have seen a marked shift towards developing a pharmacological approach to treat CF, and a number of structurally unrelated CFTR chemical activators have been discovered (Schultz *et al*., 1999; Kunzelmann & Mall, 2001). More recently, a combinatorial compound library was generated using two of these 'lead' CFTR openers, the isoflavone genistein (Illek *et al*., 1995) and the benzoquinolizinium MPB-07 (Becq *et al*., 1999), and by high throughput screening, several novel and more potent CFTR activators were identified (Galietta *et al*., 2001). Of course the ultimate goal of this research is to identify chemicals that can 'fix' CF cells, and this obviously will be helped by drugs that can activate 'mutant' CFTR. But here the problems start. There are more than 1300 different disease-causing CF mutations (http://www.genet.sickkids.on.ca/cftr/) and 'mutation-specific' drugs are likely to be required to successfully treat all CF patients. In this issue of *BJP*, Zegarra-Moran *et al*. (2002), address this problem by evaluating the effects of three chemically unrelated drugs – well-touted as CFTR activators – on their ability to stimulate a common CFTR missense mutation, G551D. This is the third most common mutation with a worldwide frequqency of ~3.0%. The replacement of aspartate for glycine occurs in the 4th amino acid of the conserved 'linker' region in NBD1, and produces a protein that is correctly inserted into the plasma membrane but cannot be activated by normal stimuli acting through the PKA cascade. This regulatory defect results not from a failure to phosphorylate CFTR, but from a gating defect due to a significantly reduced affinity of the channel for ATP (Howell *et al*., 2000). Illek *et al*. (1999) first showed that this mutant could be activated by treatment with genistein *in vitro* and probably *in vivo* as assessed by electrical nasal potential difference measurements in CF patients.

To assess which of the three CFTR activators was the most potent at stimulating the G551D mutant, Zegarra-Moran (2002), expressed wild-type (WT) and mutant G551D channels in polarized Fisher Rat Thyroid (FRT) epithelial cells and studied the effect of the CFTR openers on transepithelial/transapical Cl^- transport. The results were somewhat surprising. Neither CPX nor MPB-07, both of which stimulate WT CFTR, significantly affected G551D transfected monolayers, even with cAMP prestimulation. In contrast, genistein clearly increased Cl^- transport in pretreated G551D FRT cells. At maximal concentrations (200 μM) genistein produced an increase in Cl^- transport that was ~30% of the response of WT cells, after normalizing for CFTR expression. Based on other studies, this degree of correction is likely to be of therapeutic benefit. Similar results were also obtained using cultured nasal cells derived from one CF patient who is a compound heterozygote for G511D. Overall, this work and that of Illek *et al*. (1999), suggest that

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genistein is probably the best G551D CFTR-activating drug we have. However, there are a number of important gaps in our knowledge that these studies do not address. (1) Can genistein significantly increase ASL volume to produce any clinical benefit, given that Na^+-absorption appears not to be modulated in a reciprocal manner? (2) How does genistein activate G511D-CFTR? Previous studies have shown that genistein stimulates WT CFTR by inhibiting ATPase activity at NBD2 (Howell *et al*., 2000, and references therein), which delays closure of the channel. Does a similar mechanism apply to G551D, and if so, does this provide evidence for interactions between the two NBDs? The fact that the dose-response curve for WT and CFTR were very different (Zegarra-Moran *et al*., 2002) suggests that a different/or additional mechanism may be involved. This is supported by recent studies with MPB-91, a congener of MPB-07, that was recently shown to stimulate G551D–CFTR in an NBD2-independent manner (Derand *et al*., 2001). (3) Since high concentrations of genistein can block both WT and G551D–CFTR how will therapeutic concentrations of the drug be achieved at the level of the cells *in vivo*? Additionally, as the concentration of genistein required to activate G551D channels was much higher than for WT CFTR, this may well lead to problems if used *in vivo*. Thus in order to improve efficacy, the flavonoid-based combinatorial libraries that were recently developed by the Verkman group (Galietta *et al*., 2001) will need to be screened to find a better version of genistein. Given the current pace of developments in this field it is unlikely we will have long to wait. This is also likely to hold true for the most common CF mutation (ΔF508), which is also located in NBD1. The mutation leads to early degradation of CFTR and less than 1% of the mature protein traffics to the plasma membrane. However, substantial progress has been made in identifying drugs that can overcome this trafficking defect (Dormer *et al*., 2001; Kunzelmann & Mall, 2001), and high throughput screening is well underway. One may wonder, and hope, that a new era of 'mutation-specific' designer drugs for the treatment of CF is on the way!

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