Curcumin, a Major Constituent of Turmeric, Corrects Cystic Fibrosis Defects


Cystic fibrosis is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, ΔF508, results in the production of a misfolded CFTR protein that is retained in the endoplasmic reticulum and targeted for degradation. Curcumin is a nontoxic Ca–adenosine triphosphatase pump inhibitor that can be administered to humans safely. Oral administration of curcumin to homozygous ΔF508 CFTR mice in doses comparable, on a weight-per-weight basis, to those well tolerated by humans corrected these animals’ characteristic nasal potential difference defect. These effects were not observed in mice homozygous for a complete knockout of the CFTR gene. Curcumin also induced the functional appearance of ΔF508 CFTR protein in the plasma membranes of transfected baby hamster kidney cells. Thus, curcumin treatment may be able to correct defects associated with the homozygous expression of ΔF508 CFTR.

The ΔF508 CFTR mutation accounts for ~69% of all cystic fibrosis (CF) alleles, and ~90% of CF patients carry at least one copy of ΔF508 CFTR (1). Although it is functional as a chloride channel (2), the misfolded ΔF508 CFTR protein is retained in the endoplasmic reticulum (ER) through interactions with elements of the ER’s quality control chaperone machinery and targeted for subsequent degradation in the proteasome (3–5). Many ER lumen chaperones are calcium-binding proteins (6, 7), suggesting the possibility that treatments capable of reducing the concentration of calcium in the ER lumen might interfere with chaperone function and thus permit ΔF508 CFTR to escape. Incubation of human cell lines expressing ΔF508 CFTR with sarcoplasmic/endoplasmic reticulum calcium pump (SERCA) inhibitors results in the release of the ER-retained ΔF508 CFTR protein, permitting it to achieve its characteristic functional residence at the cell surface (8). To identify nontoxic compounds that may work through similar mechanisms, we tested the capacity of curcumin, a relatively low-affinity SERCA pump inhibitor (apparent $K_i$ ~ 5 to 15 μM (9–11)), to correct aspects of the CF defect in cell lines and mice expressing ΔF508 CFTR.

To determine whether oral curcumin treatment could safely effect the functional redistribution of the ΔF508 protein in the context of a living CF-affected organism, gene-targeted mice homozygous for the ΔF508 mutation were given 45 mg of curcumin per kilogram of body weight by mouth daily for 3 days. The dose administered was chosen to approximate, on a weight per weight basis, curcumin doses that have been well tolerated in humans in previous studies (12). The curcumin was given either as a once per day bolus or as a divided dose three times daily. Following treatment, the membrane potential difference across the nasal epithelia [nasal potential difference (NPD)] was measured (13). In untreated CF-affected animals the nasal epithelium exhibited a large, luminal-negative potential that was sensitive to amiloride, reflecting electrogenic Na+ absorption (Fig. 1A) (8, 14). Removal of luminal Cl– and exposure to isoproterenol did not substantially alter the potential in untreated ΔF508 animals (15, 16). After curcumin treatment, the average baseline NPD decreased from ~27.9 ± 0.77 mV to ~10.8 ± 0.62 mV, approaching the values in wild-type mice (~8.36 ± 0.55 mV). In addition, perfusion with the low Cl– solution and subsequent addition of isoproterenol each resulted in hyperpolarizations of the NPD similar to those seen with wild-type animals (Fig. 1A). Thus, there was a correction of both the baseline and isoproterenol-stimulated components of the NPD trace after treatment with orally administered curcumin. In contrast, phenylbutyrate treatment of the CF mice produced an effect only on the isoproterenol-stimulated component, consistent with what has been observed in human clinical trials (17) (fig. S1).

To test the specificity of these effects for ΔF508 CFTR, curcumin was administered to homozygous CFTR knockout mice (18, 19). These animals do not express any CFTR protein but retain the remaining complement of transport systems that is associated with epithelial fluid and electrolyte secretion. We found that curcumin treatment did not correct the abnormal NPD measured in the CFTR knockout mice (Fig. 1B).

To assess whether curcumin altered intestinal ion transport, we measured the rectal potential difference (RPD) in ΔF508 CF mice before and after treatment with curcumin. RPD measurements obtained from ΔF508 CF mice differ in two major characteristics from those derived from wild-type littermates (20). First, the baseline RPD of the ΔF508 CF mice is less negative than the baseline RPD of the wild-type mice. Furthermore, the RPD of wild-type mice, but not ΔF508 CF mice, hyperpolarizes in response to forskolin. The lack of response in the CF animals is believed to result from the absence of functional CFTR in the rectal mucosa. We found that after treatment with curcumin, the CF mice had a 4.46 ± 1.0 mV hyperpolarization in response to forskolin (Fig. 2A). This response is approximately 91% of the response observed in the wild-type and heterozygote mice examined in this study.

We examined the processing of ΔF508 CFTR protein expressed in the baby hamster kidney (BHK) cell line. The ER-retained ΔF508 CFTR protein is core-glycosylated (4), whereas wild-type CFTR acquires complex glycosylation (4, 21). Incubation of BHK cells expressing
The ΔF508 CFTR protein in curcumin-treated cells was confirmed through surface labeling experiments performed with BHK cells that express CFTR proteins carrying triple hemagglutinin (HA) tags in their fourth extracellular loops. Intact proteins carrying triple hemagglutinin (HA) formed with BHK cells that express CFTR directed against HA, followed by 125I-conjugated secondary antibody. Curcumin treatment resulted in the surface expression of a quantity of ΔF508 CFTR protein that was ~25% of that which could be achieved through low-temperature incubation (Fig. 3B). The functional competence of this cohort of surface ΔF508 CFTR was established by measuring iodide efflux in BHK cells expressing ΔF508 CFTR (21, 22). The peak rate of cyclic adenosine monophosphate (cAMP)–stimulated iodide efflux was ~40% higher in curcumin-treated cells than it was in cells treated with dimethyl sulfoxide (DMSO) vehicle alone (Fig. 3C). These effects were not observed in cells expressing the wild-type CFTR protein. Thus, curcumin treatment is able to induce the functional plasma membrane localization of the ΔF508 CFTR protein.

The ΔF508 CFTR protein interacts with the ER chaperone protein calnexin, which may play an important role in its ER retention (23). To determine whether curcumin treatment influences this interaction, we performed coimmunoprecipitation studies. Calnexin was readily detected in immunoprecipitates prepared from the ΔF508 CFTR-expressing Chinese hamster ovary (CHO) cells (24). Calnexin did not appear to coprecipitate with the ΔF508 CFTR protein when the CHO cells were treated with 50 μM curcumin for 3 hours before cell lysis (Fig. 3D). Thus, curcumin’s capacity to release a cohort of ΔF508 CFTR from the ER correlated with the dissolution of the calnexin–ΔF508 CFTR interaction.

Homozygous ΔF508 CFTR mice are extremely susceptible to gastrointestinal obstruction, leading to considerable mortality. It has been shown that the osmotic laxative Colyte (Schwarz Pharma, Milwaukee, WI) markedly increases these animals’ survival rate (25). We treated homozygous ΔF508 CFTR mice with oral curcumin and compared their survival to mice given no treatment or the standard Colyte treatment. Six of the ten mice in the no-treatment group died of intestinal obstruction within 10 weeks (Fig. 2B). This 60% mortality rate is similar to values previously reported for this CF mouse model (26). Only one mouse in each of the Colyte- (n = 10) and curcumin-treated groups (n = 10) died. The Colyte-treated mice gained 0.99 ± 0.47 g of weight per week, which was similar to the 0.77 ± 0.13 g/week gained by the curcumin-treated group. These gains can be contrasted to the average 0.40 ± 0.43 g/week of weight loss observed in the untreated mice (Fig. 2C).

Our previous work has demonstrated that several other structurally diverse SERCA pump inhibitors, including thapsigargin and 2,5-di-(tert-butyl)-1,4-hydroquinone (DBHQ),
share curcumin’s ability to induce the functional expression of the ΔF508 CFTR protein, both in vitro and in vivo (8). A number of ER chaperone polypeptides are calcium-binding proteins (6, 7), which suggests that these compounds may exert their effects by altering the concentration of free calcium in the ER lumen, thus perturbing the capacity of calcium-dependent chaperone mechanisms to recognize and retain the misfolded ΔF508 CFTR protein. It is worth noting, however, that curcumin exhibits structural similarities to isoflavonoid compounds that may bind directly to the CFTR protein and alter its channel properties (27). It is also possible, therefore, that curcumin may bind directly to CFTR and that such a direct interaction may stabilize its tertiary structure, thus permitting it to evade the ER quality-control machinery.

Human studies indicate that curcumin is tolerated in extremely large oral doses without apparent toxicity (12). The dose employed in the present animal experiments corresponds, on the basis of mg/kg scaling, to doses of commercially available curcumin products that are routinely consumed. This extensive experience with curcumin, both in animal models and in patients, coupled with its apparent lack of adverse effects, could facilitate the translation of the data presented here to a human clinical trial. It must, of course, be noted that the success obtained in the present animal experiments provides no guarantee that similar results will be obtained with curcumin in CF patients. Issues relating to curcumin’s bioavailability, to speciesspecific pathways through which it may be metabolized, and to the extent to which the mouse model accurately recapitulates relevant features of the CF phenotype could all diminish curcumin’s potential to alter ΔF508 CFTR function in the setting of the human disease. The data presented here, however, suggest that curcumin and curcumin derivatives represent promising new candidate compounds that may prove useful in the search for small-molecule pharmacotherapies for CF and for other protein-folding diseases.

Fig. 3. Curcumin promotes the accumulation of mature ΔF508 CFTR in BHK cells. (A) Detection of curcumin-induced accumulation of complex-glycosylated ΔF508 CFTR by immunoblotting. BHK cells expressing the HA-tagged ΔF508 CFTR were treated with the indicated concentrations of curcumin for 16 hours. Cells expressing wild-type CFTR and cells expressing ΔF508 CFTR that had been incubated at reduced temperature (26°C) for 16 hours were included as controls. Immunoblotting for Na/K-ATPase α subunit was performed to ensure equal loading. (B) Cell surface density of ΔF508 CFTR. BHK cells expressing ΔF508 CFTR tagged with the 3HA epitope in its fourth extracellular loop were incubated in the presence of curcumin (5 μM) or DMSO (control) at 37°C or at 26°C for 16 hours. Cell surface density of CFTR was measured by antibody to HA and by 125I-labeled secondary antibody. Data are expressed as the percentage of specific antibody binding per milligram of protein measured at 37°C. Data are means ± SEM; n = 3. (C) Iodide conductance of ΔF508 CFTR–expressing BHK cells. BHK cells were incubated in the presence of curcumin (5 μM) or DMSO (control) at 37°C or at 26°C for 16 hours. Activation of the ΔF508 CFTR channels was achieved by the addition of the cAMP-agonist cocktail (+cAMP: 20 μM forskolin, 0.2 mM 3-isobutyl-1-methyloxanthine [IBMX], and 0.5 mM chlorophenylthio-cAMP) at the arrow to iodide-loaded cells. Iodide efflux was measured with an iodide-selective electrode and normalized for protein content. Data are means ± SEM from two to four independent experiments. The basal iodide efflux was depicted only for DMSO-treated cells, because similar results were obtained for all the other conditions. Stars indicate that the amount of iodide efflux for curcumin and 26°C treated cells is significantly ($P < 0.05$) greater than that measured in DMSO-treated cells. (D) Curcumin effect on ΔF508 CFTR–calnexin interactions. CHO cells expressing ΔF508 CFTR were incubated for 3 hours at 37°C with DMSO or with 50 μM curcumin, after which they were lysed and subjected to immunoprecipitation with antibodies to CFTR. The immunoprecipitated proteins (IP) and aliquots of the lysates (L) were analyzed by Western blotting using an antibody directed against calnexin.

References and Notes
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Supporting Online Material
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Materials and Methods
Table S1
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