Ibuprofen regulation of microtubule dynamics in cystic fibrosis epithelial cells

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Cystic fibrosis (CF) is an autosomal recessive disease that is characterized in part by aggressive pulmonary inflammation (43). CF inflammation is the likely result of both chronic bacterial infection providing an overwhelming inflammatory stimulus and inherent changes in CF cell biology (1, 2). Several alterations to pathways regulating inflammatory responses have been reported in CF such as endoplasmic reticulum stress responses and loss of anti-inflammatory mediators (i.e., lipoxin) (18, 19). We have demonstrated deficiencies in IFN-γ signaling and elevated small GTPase RhoA expression and activity in CF cells as consequences of impaired intracellular transport regulation, which are potential mediators of inflammatory dysregulation (20, 27, 44). Multiple cell types have also been implicated in the CF inflammatory response. Most studies have focused on epithelial responses to bacteria and bacterial products, but recent evidence also strongly implicates macrophage dysfunction as a source of aberrant immune response in CF (3–5).

The importance of controlling inflammation in CF as a therapeutic approach has already been demonstrated. The use of high-dose ibuprofen, especially in children, has been shown to slow the decline of lung function as measured by FEV₁ and help patients gain or maintain body weight in multiple studies (24–26, 28, 29). However, potential adverse side effects have limited the use of ibuprofen to where only ~3% of CF patients utilize the therapy (9a). New CFTR-targeted therapies, such as ivacaftor, have tremendous benefit in patients carrying a G551D CFTR allele but do not seem to be able to effectively control inflammation. A recent study examining subjects after 6 mo of ivacaftor treatment found no improvement in the levels of inflammatory mediators (37). Even with the advent of CFTR-targeted therapies, it is likely that effective anti-inflammatory therapies will still be needed.

Currently, the only anti-inflammatory treatment recommended for CF patients is high-dose ibuprofen (24–26, 28, 29). In addition to the potential adverse effects of the therapy, there is also a lack of clarity as to how ibuprofen works because the dosage necessary for benefit in CF far exceeds that needed for cyclooxygenase inhibition (35). Identifying the mechanism of high-dose ibuprofen efficacy would clarify pathways associated directly with adverse inflammatory responses in CF and illuminate therapeutic targets for new therapies that could replicate the benefits of ibuprofen therapy without the associated adverse effects.

We recently identified altered microtubule dynamics in CF cells, including reduced microtubule acetylation (40) and slower rates of microtubule formation (41). Microtubule structure and function are factors known to contribute to inflammatory response regulation (46). One study has demonstrated that enhancing microtubule stability by means of taxol attenuates inflammation after endotoxin-induced inflammation using a mouse lung injury model (32). It has also been shown that increasing microtubule acetylation by histone deacetylase (HDAC) 6 inhibition leads to enhanced anti-inflammatory signaling characterized by stimulation of interleukin (IL)-10 production (42). Specifically in CF epithelial cells, we have shown that restoring microtubule acetylation levels through HDAC6 inhibition inhibits NF-κB signaling (40).

In this study, we explore the hypothesis that the efficacy of ibuprofen as a CF therapy may be due to an influence of ibuprofen on microtubule regulation. It is identified here that ibuprofen increases both the rate of microtubule formation and the stability of microtubules in CF cells. The impact of ibuprofen on microtubules can be seen functionally by the improvement observed in cholesterol processing as a measure of intracellular transport. Mechanistically, AMPK activation is a candidate as a mediator of the effects of ibuprofen on microtubules. These findings demonstrate a novel mechanism of action for ibuprofen, clarify a mechanism of efficacy in CF, and illustrate a potential mechanism to explain the antiproliferative properties of ibuprofen.

**METHODS**

**Cells and materials.** All cells were grown at 37°C in 5% CO₂ incubators, unless noted. IB3-1 cells, human epithelial with the ΔF508 mutation (CF phenotype), and S9 cells, IB3-1 cells stably transfected with the full-length wild-type (WT) CFTR (control),
were a generous gift from Pamela L. Zeitlin (Johns Hopkins University, Baltimore, MD). Cells were cared for as previously described in LHC-8 basal medium (Biofluids, Camarillo, CA) supplemented with 5% FBS and 1 U/ml penicillin-streptomycin (38). Human nasal epithelial cells were obtained from the Case Western Reserve University (CWRU) CF Center cell culture core facility. None of the working cell lines were authenticated or tested from mycoplasma recently.
In vitro microtubule formation assay. Cells were grown on collagen-coated coverslips to 75–80% confluency. Cells were removed from the 37°C 5% CO₂ incubator and placed on ice for 30–60 min. After depolymerization period, prewarmed 37°C media (with vehicle or drug) was added at designated time points (0–20 min). At the end of the time course, cells were rinsed with PBS and fixed at the indicated time points. Cells were immunostained according to the protocol described below.

Cells were visualized in the appropriate range on a Leica DM6000 microscope (×40 oil objective) with Improvision’s Velocity software. For each time point, 5–10 representative fields were captured, yielding 40–80 cells. Each cell was scored as having or not having an aster present, and quantification was determined by the ratio of cells with aster/microtubule formation to total cells at various time points. In the figure legends, n represents the number of times each experiment was repeated per condition, yielding reproducibility of the experiment.

Immunostaining. Antibodies against α-tubulin (Abcam ab15246) were obtained. Anti-rabbit IgG Fab2 Alexa Fluor 594 Molecular Probes antibodies were obtained from Cell Signaling (8898S). Cells were rinsed three times with PBS and fixed and permeabilized with acetone for 20 min at −20°C. Cells were rinsed with PBS and then blocked with 5% goat serum in PBS for 30–60 min rocking at room temperature. Primary antibodies were diluted in 5% goat serum in PBS and were added for 1 h rocking at room temperature. Cells were rinsed three times with PBS and then incubated with secondary antibodies in 5% goat serum in PBS. Cells were mounted with SlowFade Gold Antifade (Invitrogen) on slides. Cells were visualized in the appropriate range using a Leica DM6000 upright microscope (×40 oil objective) with Improvision’s Velocity software.

Microtubule extension assay. Cells were grown on collagen-coated coverslips at 37°C 5% CO₂ to 75–80% confluency and subjected to immunofluorescence staining with α-tubulin to visualize microtubules. Cells were imaged on Leica TCs SP8 confocal (×64 oil objective) with Leica LAS software. For microtubule quantification, cell boundaries were outlined and the concentric outline shape was reduced to 70% to demarcate peripheral (outer 30% of diameter) and central (inner 70% of diameter) regions. Integrated density in the peripheral area was measured with ImageJ and was calculated as a percentage of the integrated density of the entire cell area. Results were normalized in each experiment.

Filipin staining. Cells were plated at a density of 25,000–50,000 cells/well and grown to 60–70% confluency on Fisher brand coverslips. Cells were rinsed twice with PBS and then fixed with 4% paraformaldehyde for 30 min. Cells were rinsed twice more with PBS and then incubated with 0.05 mg/ml Filipin (Sigma-Aldrich, St. Louis, MO) in PBS for 1 h on a shaker in the dark. Filipin was dissolved freshly in dimethylsulfoxide before each experiment. Cells were rinsed in PBS before being mounted with SlowFade Gold Antifade reagent on slides. Cells were visualized in the ultraviolet range on a Leica DM6000 microscope (×40 oil objective) with Improvision’s Velocity software.

Western immunoblotting. Antibodies against α-tubulin (ab15246) were obtained from Abcam (Cambridge, MA). Antibodies against acetylated α-tubulin (sc23950) were obtained from Santa Cruz (Dallas, TX). Actin (A2066) antibodies were obtained from Sigma-Aldrich (St. Louis, MO). Rap1 (no. 07-916) antibodies were obtained from Millipore (Billerica, MA).

Cells were grown to 95% confluency and lysed with lysis buffer (50 mM Tris pH 7.5, 1% Triton X-100, 50 mM NaF, 200 mM Na3VO4, and 10 μg/ml pepstatin and leupeptin) for 30 min at 4°C. Lysates were centrifuged at 10,000 rpm for 5 min. Proteins were separated with SDS-PAGE containing 20–60 μg protein on a 7.5%–12% polyacrylamide gel. Samples were transferred to an Immobilon-P membrane (Millipore, Bedford, MA) at 15 V for 30 min. Blots were blocked in 10% nonfat dehydrated milk in PBS (138 mM NaCl, 15 mM Na2HPO4, 1.5 mM KCl, and 2.5 mM KH2PO4) with 0.1% Tween-20 (PBS-T) for 45 min at room temperature and then incubated with primary antibodies (1:1,000 dilution) in 10% nonfat dehydrated milk in PBS-T overnight at 4°C. Blots were washed three times for 10 min each with PBS-T, incubated with the respective secondary antibodies conjugated to horseradish peroxidase (1:3,000 dilution) in PBS-T, washed again with PBS-T, and visualized with SuperSignal chemiluminescent substrate (Fierce, Rockford, IL) and the Chemidoc Imaging System (Bio-Rad, Hercules, CA). Quantification of protein expression was performed with Quantity One (Bio-Rad) software.

Rap1 activation. Using the cell models described above, we assessed Rap1 activation by the Rap1 activity assay with a kit obtained from Millipore according to manufacturer instructions. Rap1-GTP and total Rap1 protein content was determined by Western blot analysis. ChemiDoc imaging system (Bio-Rad) was used to image gels and quantification of protein expression was accomplished by densitometry software (Quantity One; Bio-Rad).

Statistics. Sample size was chosen on the basis of previous experience. No blinding was used in any sample collections nor were samples randomized. All experiments were conducted contracting three or more independent experiment groups; thus significance was determined for all experiments by one-way ANOVA (unless noted in figure legend). Comparisons between groups were performed with the Newman-Keuls post hoc test. Data were normally distributed in each group being compared and had similar variance. All data are expressed as means ± SE.

RESULTS

Ibuprofen stimulates microtubule reformation in CF cells. To begin testing the hypothesis that ibuprofen may influence microtubule regulation, the effect of ibuprofen treatment on microtubule formation rates was examined. Microtubule dynamics after ibuprofen treatment were examined by observing microtubule reformation rates after complete solubilization of tubulin. Formation is quantified by determining aster formation over time as previously described (10, 41). CF-model IB3 and CFTR-corrected control S9 cells were treated with high-dose ibuprofen (500 μM) for 24 h. CF cells showed slower rates of microtubule formation compared with control cells consistent with previous results (41). By 4 min, the ratio of cells with asters to total cells in ibuprofen-treated CF cells was 0.71 ± 0.05.
where cells left untreated were found to have a ratio of 0.30, and cells left untreated were found to have a ratio of 0.09, where cells left untreated were found to have a ratio of 0.30 ± 0.07. The significant increase in aster formation was maintained at 8 and 12 min in ibuprofen-treated CF cells, equaling rates seen in control cells. A similar, but not statistically significant, improvement in the rate of aster formation was also observed in the control cells (Fig. 1, A and B). All groups reached maximal aster formation by 20 min as previously reported (41). Group comparisons made at 8 min showed aster formation in ibuprofen-treated CF cells was equal to that in control cells (Fig. 1C). Ibuprofen enhances microtubule formation in IB3 cells in a dose-dependent manner (Fig. 1D).

As a functional measure of microtubule correction, we examined cholesterol transport in CF cells in response to ibuprofen treatment. We and others have previously shown CF cells do not process cholesterol correctly, resulting in perinuclear accumulation of free cholesterol (16, 30, 31, 45). This perinuclear accumulation is due to impaired microtubule-mediated transport, and the correction of microtubule function by either increasing tubulin acetylation or improving rates of formation results in the dispersal of perinuclear cholesterol in CF cells (40, 41). Consistent with these findings, ibuprofen restored cholesterol trafficking in the CF IB3 cells, supporting the hypothesis that ibuprofen treatment normalizes microtubule functioning (Fig. 1E).

Effect of other cyclooxygenase inhibitors on microtubule formation. We then examined whether the impact on microtubules is specific for ibuprofen or whether other cyclooxygenase inhibitors have the same effect. Cells were treated with aspirin (ASA, 500 μM), celecoxib (10 μM), or, as an unrelated analgesic control, acetaminophen (APAP, 500 μM), for 24 h. Although celecoxib may have slightly enhanced microtubule formation rates, no compound significantly enhanced the microtubule reformation rates of IB3 cells compared with vehicle control with no drug treatment (NT) (Fig. 2, A–D). In this experiment, all tested compounds were water soluble so no solvent vehicle control was needed. Since celecoxib had a partial effect, we performed a dose curve to determine whether a higher dose could have a more distinct impact. We were only able to achieve a dose of 50 μM due to solubility and toxicity limits. No further enhancement in microtubule formation was observed at any dose (Fig. 2E). Though there is still a concentration difference between ibuprofen and celecoxib, the IC50 of ibuprofen toward COX-2 is 100 μM compared with only 0.04 μM for celecoxib (11). Therefore, both drugs are used in significant excess of their required doses for COX-2 inhibition and it is unlikely that cyclooxygenase inhibition is the mechanism of ibuprofen efficacy.
Ibuprofen directly impacts microtubule extension and structure. In addition to increased rates of formation, it was observed that ibuprofen treatment had a structural effect on microtubules, both straightening and lengthening them compared with untreated IB3 cells. This effect on microtubule structure was not observed in our previous work where specifically activating EPAC1 with 8-cpt-2-O-Me-cAMP increased microtubule formation rates in CF cells (41), suggesting that another pathway is likely impacted by ibuprofen. To quantify whether microtubule morphology is affected by ibuprofen, control and IB3 cells treated with either ibuprofen or vehicle were immunostained for α-tubulin to determine whether ibuprofen impacted the amount of microtubules that could extend to the periphery by a technique previously described (10).

Quantitative analysis of microtubule structure revealed that expanded peripheral microtubule networks are present following ibuprofen treatment. CF cells had 21% fewer microtubules present in the periphery compared with control S9 cells (Fig. 3A). Ibuprofen treatment restored CF peripheral microtubule content to control levels, demonstrating a clear effect not only on microtubule formation rates but also on morphology [Fig. 3A, top (whole cell) and bottom (higher magnification inset)]. There was no observable influence on microtubule structure after treatment with aspirin, celecoxib, or acetaminophen (Fig. 3B).

Ibuprofen stimulates microtubule formation in primary human nasal epithelial cells. To confirm that ibuprofen acts similarly in primary cells, human nasal epithelial (HNE) cells were
obtained from three subjects homozygous for the F508del CFTR genotype. Our previous work demonstrated reduced rates of microtubule formation in CF primary HNE cells compared with healthy controls, suggesting that this cell system would be appropriate to test the effects of ibuprofen (41). Samples were obtained by nasal brushing and expanded in culture to perform a microtubule reformation assay as previously described (41). Cells were treated with either vehicle or ibuprofen for 24 h and analyzed for microtubule formation differences. Although base rates of microtubule formation are faster in HNE cells compared with cultured cell models, CF rates of formation were slower than WT, consistent with previous results. The addition of ibuprofen for 24 h to F508del HNE cells normalized microtubule formation rates (Fig. 4, A and B).

Ibuprofen does not activate EPAC1/Rap1 or impact microtubule acetylation. Our previous work identified two microtubule-directed interventions that could correct intracellular transport in CF cells: increasing microtubule acetylation by HDAC6 inhibition and restoring formation rates by EPAC1-selective activation (40, 41). To determine whether ibuprofen interacts with either of these pathways, the impact of ibuprofen treatment on acetylated tubulin levels and EPAC1/Rap1 activation was assessed. Control S9 and CF-model IB3 cells were treated for 24 h with ibuprofen (500 μM) and analyzed for changes to acetylated tubulin levels and EPAC1/Rap1 activation. Control and F508del/F508del HNE cells subjected to microtubule polymerization assay (0–8 min), and immunostained for α-tubulin. Representative images are shown (3 biological replicates of each treatment group). B: quantified microtubule polymerization data are shown from F508del/F508del and control human nasal epithelial cells’ microtubule polymerization assay. The ratio of asters formed to total cells was quantified and plotted as a function over time (0–8 min) (control vs. F508del/F508del, n = 3, *P < 0.05; F508del/F508del vs. F508del/F508del + Ibp, n = 3, °P < 0.05; 1-way ANOVA with Newman-Keuls post hoc test). Ibp, ibuprofen.
other mechanisms still need to be elucidated. Likely involved in a portion of the response to ibuprofen, but tubules. These data demonstrate that the AMPK pathway is because of background fluorescence from fragmented microtubules. Results in the presence of compound C could not be quantified (Fig. 6 E). Microtubule extension in microtubule fragmentation (Fig. 6 D) but does disrupt microtubule extension, resulting in microtubule fragmentation (Fig. 6 E). Microtubule extension results in the presence of compound C could not be quantified because of background fluorescence from fragmented microtubules. These data demonstrate that the AMPK pathway is likely involved in a portion of the response to ibuprofen, but other mechanisms still need to be elucidated.

Ibuprofen had no impact on microtubule acetylation, suggesting ibuprofen is directly affecting microtubule formation rates (Fig. 5 A). We previously reported that reduced EPAC1 function is responsible for slower microtubule formation in CF cells. EPAC1 also functions as Rap1 guanine nucleotide exchange factor (Rap1GEF) (12). To evaluate EPAC1 activation, whether an inhibitor of AMPK, compound C (10 μM), could block the efficacy of ibuprofen. Results demonstrated that one alternative pathway through which ibuprofen can act is the activation of AMPK (22, 39). We tested whether AMPK activation with the AMP analog 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) could mimic the effects of ibuprofen on microtubule regulation in CF model cells. Like ibuprofen, AICAR (500 μM, 24 h) was able to completely restore microtubule elongation rates in CF IB3 cells to control levels (Fig. 6, A and B). This finding is consistent with previous work showing the impact of AMPK activation on microtubule polymerization through activation of Clip170 (34). Also similar to ibuprofen, AICAR had no effect on microtubule acetylation when S9 and IB3 cells were treated for 24 h with AICAR (500 μM) (Fig. 6 C). Since AMPK activation could mimic the effect of ibuprofen, it was tested whether an inhibitor of AMPK, compound C (10 μM), could block the efficacy of ibuprofen. Results demonstrated that compound C fails to block aster formation induced by ibuprofen (Fig. 6 D) but does disrupt microtubule extension, resulting in microtubule fragmentation (Fig. 6 E). Microtubule extension results in the presence of compound C could not be quantified because of background fluorescence from fragmented microtubules. These data demonstrate that the AMPK pathway is likely involved in a portion of the response to ibuprofen, but other mechanisms still need to be elucidated.

Since ibuprofen effects on microtubules are able to functionally improve intracellular transport as marked by improved cholesterol processing, we used correction of cholesterol accumulation in CF cells as a means to quantify the role of the AMPK pathway in mediating ibuprofen effects. CF IB3 cells and control S9 cells were treated with ibuprofen (500 μM) with and without the AMPK inhibitor compound C (10 μM). In IB3 cells, ibuprofen reduced the percentage of cells accumulating cholesterol from 63.8 ± 2.4% in vehicle-treated cells to 24.5 ± 7.1% (Fig. 7, A and B). The presence of compound C completely blocked the ability of ibuprofen to reduce cholesterol accumulation (59.9 ± 8.3% of cells showing accumulation). Interestingly, in control S9 cells the presence of compound C increased cholesterol accumulation from 20.1 ± 4.9% of cells to CF-like levels of 65.5 ± 7.2% of cells showing cholesterol accumulation.

AMPK activation with AICAR (500 μM) mimicked the effect of ibuprofen on microtubule formation rates in CF cells (Fig. 6). Here, we assessed whether AICAR could also replicate the impact of ibuprofen on the functional outcome of alleviating cholesterol accumulation. Like ibuprofen, AICAR reduces cholesterol accumulation from 69.9 ± 1.5% of cells showing accumulation in vehicle-treated cells to 18.7 ± 10.4% of cells (Fig. 7 C). The AMPK inhibitor blocked the effects of AICAR on cholesterol accumulation (67.1 ± 3.3% of cells showing cholesterol accumulation). These data show that both ibuprofen and AICAR have identical effects on cholesterol processing in CF model cells and that the effects of both are equally blocked by the AMPK inhibitor compound C, strongly suggesting that AMPK signaling is vital to the effects mediated by ibuprofen on microtubule structure and function.

**DISCUSSION**

In the clinic, the efficacy of high-dose ibuprofen in the treatment of CF has been shown in multiple studies (24–26, 28,
However, the risk of adverse effects limits its use to only \( \sim 3.3\% \) of patients (9a). There is also a lack of clarity as to how ibuprofen elicits its effects as the doses necessary for therapeutic benefit far exceed those needed to cyclooxygenase inhibition (35). Understanding how ibuprofen works could lead to the development of new therapeutics that avoid the associated adverse effects. Here, we identify a novel mechanism of action of ibuprofen, where microtubule formation rates are enhanced.
restored in primary human nasal CF epithelial cells as well as an immortalized CF cell model. This restoration of microtubule alterations we have previously identified in CF also leads to correction of intracellular transport as measured by cholesterol processing. As we have shown previously, these phenotypes are associated with aspects of CF-related inflammatory signaling (20, 27, 30, 31, 40, 41, 44, 45).

Mechanistically, AMPK activation with AICAR is able to completely replicate the effects of ibuprofen in CF cells and the AMPK inhibitor compound C blocks microtubule reformation in the presence of ibuprofen, although compound C has no impact on aster formation. If further studies confirm that the mechanism of action of ibuprofen in CF cells is through the AMPK pathway, then AMPK agonists may be considered as an alternative therapy to ibuprofen with fewer adverse side effects. AMPK activation has been proposed previously as a potential anti-inflammatory therapy for CF. It was initially identified by Hallows et al. (17) that AMPK activity is increased in the absence of CFTR function likely as an adaptive response to heightened inflammatory signaling.

Fig. 6. AMPK regulation of microtubule dynamics. A: aster reformation was analyzed in CF (IB3) and wild-type (S9) cells treated with either AICAR (500 μM) or vehicle for 24 h. Immunofluorescence analysis for α-tubulin was performed after depolymerizing microtubules on ice and restimulating growth (0–20 min). Representative images of 4 experiments are shown. B: quantification of aster formation after high-dose ibuprofen treatment was determined by the ratio of cells with perinuclear cholesterol accumulation after (B) 500 μM ibuprofen and/or 10 μM compound C and (C) 500 μM AICAR and/or 10 μM compound C. Filipin staining was quantified (bottom) by determining the percent of cells with perinuclear cholesterol accumulation. Significance was determined by 1-way ANOVA with Newman-Keuls post hoc test comparing responses at each dose. Significance between S9 NT and IB3 NT was determined (*P < 0.05) by Student’s t-test.

Fig. 7. Intracellular transport is disrupted after AMPK inhibition. A: S9 and IB3 cells were treated with vehicle, 500 μM ibuprofen, 500 μM AICAR, and/or 10 μM compound C (CC) for 24 h and stained with filipin. Representative images of 3 experiments are shown. B and C: filipin staining was quantified by determining the percent of cells with perinuclear cholesterol accumulation after (B) 500 μM ibuprofen and/or 10 μM compound C and (C) 500 μM AICAR and/or 10 μM compound C. Filipin staining was quantified (bottom) by determining the percent of cells with perinuclear cholesterol accumulation. Significance was determined by 1-way ANOVA with Newman-Keuls post hoc test comparing responses at each dose. Significance between S9 NT and IB3 NT was determined (*P < 0.05) by Student’s t-test.

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hancement of AMPK activity in WT and CF primary bronchial epithelial cells reduced inflammatory responses, suggesting that AMPK activation could be a viable anti-inflammatory approach. Another study demonstrated that AMPK activation in CF cells increased airway surface fluid height in addition to attenuating inflammatory responses, providing a dual benefit (33). However, multiple studies have shown that AMPK activation potently inhibits CFTR-mediated chloride transport (21, 23). Reports are mixed as to the effect ibuprofen has on CFTR function, suggesting AMPK activation is not the only mechanism by which ibuprofen may be acting. Identifying the common intermediates linking ibuprofen and AMPK signaling to microtubule regulation may help identify a new anti-inflammatory approach for the treatment of CF.

Although this study focuses on the effects of ibuprofen treatment on microtubule regulation in CF cells, these findings potentially have much broader implications. It is hypothesized that microtubule disruption through genetic or chemical induction contributes to Parkinson’s disease (PD) pathophysiology (6, 7, 13, 36). Furthermore, multiple studies demonstrate that ibuprofen use is associated with a significant decrease in risk for developing PD (8, 14, 15). Likewise, ibuprofen has been shown to have protective effects in Alzheimer’s disease where ibuprofen protected against hippocampal volume changes and N-acetylaspartate loss in aged mice (17–23 wk) (9). The mechanism by which these benefits occur is unknown, but our results suggest that ibuprofen effects on microtubule formation rates should be explored in other disease models.

In summary, our results define a novel effect of ibuprofen treatment on CF cells. The stabilizing effect of ibuprofen treatment on microtubules reverses a cellular phenotype we have previously described that directly impacts intracellular transport. The effect is unlikely solely due to cyclooxygenase inhibition as neither aspirin nor celecoxib are able to elicit the same response, however, the AMPK pathway is likely a key mechanism by which these benefits occur. AMPK activation could be a viable anti-inflammatory strategy by which these benefits occur in CF cells. The stabilizing effect of ibuprofen treatment on CF cells may explain the broader benefits of ibuprofen treatment on CF cells increased airway surface fluid height in addition to attenuating inflammatory responses, providing a dual benefit (33). However, multiple studies have shown that AMPK activation potently inhibits CFTR-mediated chloride transport (21, 23). Reports are mixed as to the effect ibuprofen has on CFTR function, suggesting AMPK activation is not the only mechanism by which ibuprofen may be acting. Identifying the common intermediates linking ibuprofen and AMPK signaling to microtubule regulation may help identify a new anti-inflammatory approach for the treatment of CF.

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In summary, our results define a novel effect of ibuprofen treatment on CF cells. The stabilizing effect of ibuprofen treatment on microtubules reverses a cellular phenotype we have previously described that directly impacts intracellular transport. The effect is unlikely solely due to cyclooxygenase inhibition as neither aspirin nor celecoxib are able to elicit the same response, however, the AMPK pathway is likely a key component of the ibuprofen-mediated response. These results help define directly the efficacy of ibuprofen as a CF treatment and suggest a mechanism to explain the broader benefits of ibuprofen treatment. Understanding how ibuprofen works in CF cells will clarify approaches to develop an alternative anti-inflammatory therapy that can avoid the adverse effects associated with its use.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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