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Early View

Research letter

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Long-term effect of CFTR modulator therapy on airway nitric oxide

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To the Editor:

The fraction of exhaled nitric oxide (FeNO) is generally lower in individuals with cystic fibrosis (CF), compared to healthy controls. Two recent studies reported that the CFTR potentiator ivacaftor, resulted in an increase in FeNO after 4 weeks therapy (1,2), suggesting that changes in FeNO have the potential to serve as biomarker of restored CFTR function. However, it is currently unknown whether ivacaftor results in a sustained increases in FeNO and whether combination therapy of ivacaftor with the CFTR corrector lumacaftor, also leads to changes in FeNO. Therefore, the objective of this research was to document long-term effects of ivacaftor and lumacaftor-ivacaftor therapy on FeNO in treated CF patients. The two prospective observational studies were approved by the local institutional review boards (Hospital for Sick Children's REB #1000036224 and #1000057599, St. Michael's Hospital REB #13-089). Patients were included if they had a confirmed diagnosis of CF and were eligible for treatment with either therapy. FeNO was measured before, and 1, 3, 6, 12 and 24 months after initiation of therapy during regular outpatient visits. Sputum samples were collected in the ivacaftor cohort. The non-liquid phase (mucus plugs) of the sputum was processed by adding 0.1% dithiothreitol in Dulbecco's phosphate-buffered saline (4:1, vol:wt), and the clear supernatant of the cell suspension was separated from the cells by centrifugation (3,4). No protease inhibitors were added. Samples were stored at -80C before analysis of L-arginine metabolism using liquid chromatography-mass spectrometry (LC-MS), as reported (5). NO metabolites nitrate and nitrite were measured in sputum by Griess reagent (6) and myeloperoxidase (MPO) by ELISA (R&D Systems, Inc., Minneapolis, MN). Changes in outcomes between visits were assessed using paired t-tests and Wilcoxon sign-rank tests for skewed distributions, and correlations with the Pearson’s correlation coefficient.

RESULTS

A total of 34 patients treated with CFTR modulator therapy were included. The ivacaftor cohort consisted of 8 pediatric (median (IQR) age 12.5 (9.4, 14.0) years, 63% female) and 12 adult (age 32.6 (24.6, 42.8) years, 58% female) patients. In the lumacaftor-ivacaftor cohort there were 14 pediatric patients (age 14.5 (14.0, 16.9) years, 57% female) and no adults.

Ivacaftor cohort

At 1 month follow up the cohort consisted of 7 children and 9 adult patients, at 1 year of 6 children and 5 adults and at 2 years, of 6 children and 4 adults. FEV_1 at baseline was 78 (71, 90) in the pediatric and 62 (47, 74) % of predicted in the adult patients. FEV_1 was improved for the total group at one month, from 69.7% to 81.1% predicted (Δ 11.4; 95% CI 6.1, 16.8; p<0.001), and remained improved for the entire follow-up. Similar results were seen for FVC, with an improvement from 88.9% to 97.8% predicted (Δ 8.9; 95% CI 5.0, 12.8; p<0.001) at 1 month, which persisted throughout follow-up.

FeNO was increased compared to baseline after 4 weeks on ivacaftor (n=16), as previously reported (1), and remained increased throughout follow-up (figure). When including all measurements, FeNO weakly correlated with FVC (r=0.29, p=0.009) and FEV_1 (r=0.41, p<0.001, n=81), but changes in FeNO did not correlate with changes in PFTs over time (r=0.19, p=0.13 for FEV_1; r=0.14, p=0.28 for FVC % predicted; n=64).

Arginine / NO metabolism in ivacaftor treated patients

A mild decreases at 1 month was seen in sputum L-arginine, a substrate for NO synthases (NOS) (baseline 14.7 (8.1, 40.7) μmol/L, median Δ -5.4, p=0.04, n=13), and an insignificant decrease for the NOS inhibitor asymmetry dimethylarginine (ADMA) (baseline 0.08 (0.01, 0.26) μmol/L, median Δ –0.01, p=0.06). While there was a correlation between FeNO and

sputum L-arginine/ADMA ratio, an index of NOS impairment (r=0.31, p=0.03, n=51), which was supportive of previous observations (5), L-arginine/ADMA remained unchanged during follow-up.

Myeloperoxidase (MPO) was decreased at 1 month (baseline 27.0 (10.7, 52.2) μg/mL median Δ-4.8, p=0.03) in the total group (n=9) but no other time point. There was no change in sputum levels of nitrite or nitrate in the total group but reduced nitrite at 4 weeks in adults (baseline 22.8 (21.0, 37.2) μmol/L median Δ –9.1, p=0.03). Nitrite/nitrate ratio was decreased from baseline at 4 weeks (p=0.06) but no other visit and in adults only (n=7). There was a significant reverse correlation between sputum MPO and FeNO (r= -0.34, p=0.02, n=45) suggesting that a decrease in MPO content may contribute to the increase in FeNO with ivacaftor therapy.

Lumacaftor-Ivacaftor cohort

Median (IQR) FEV_1 for the cohort was 80.1 (68.6, 93.8) % predicted at baseline and was not significantly different at any time point after initiation of therapy. There was also no change in FVC % predicted during follow-up.

Median FeNO before lumacaftor-ivacaftor (10 (8, 15) ppb) was similar to ivacaftor baseline values but did not change within the first year of treatment (figure). However, increased FeNO from baseline was found for 5 patients at the 2 year follow-up (median increase of 9 ppb, 95% CI 2.8, 15.6; p=0.02). Changes in FeNO did not correlate with changes in FEV_1 (r=0.14, p=0.35).

DISCUSSION

Alterations in the L-arginine/NO metabolism and reduced NO availability are thought to be clinically important in CF as low NO may contribute to airways obstruction and favour

colonization with certain pathogens. While the exact reasons for the decreased FeNO in CF are currently not known, recent studies had shown that 4 weeks of therapy with the CFTR potentiators ivacaftor resulted in an increase of FeNO (1,2,7). We here present data showing that the increase in FeNO on ivacaftor therapy is not short lived or transient but rather sustained over a 2 year follow-up. Further, using LC-MS analyses in sputum we demonstrate that the long-term increase in FeNO is not due to changes in L-arginine availability or L-arginine metabolism in the airways. However, our data suggest that changes in airway metabolism of NO by MPO may be a cause for the observed increase in FeNO.

Previous studies had shown that the relative quantities of NO, NO_2^- and NO_3^- provide mechanistic insight into NO metabolism in the CF lung and that NO catabolism within the airway prior to exhalation may contribute to reduced FeNO in CF. NO oxidized by MPO results in NO_2^- and continued oxidation by MPO and other oxidative processes in NO_3^- formation (8,9). We observed a decrease in sputum MPO levels, decreased nitrite and thus relatively more nitrate (no change in total $NO_2^- + NO_3^-$), which suggests that liberation of NO from NO_2^- stored in airway secretions may contribute to the observed increase in FeNO at 4 weeks therapy. Although changes in MPO were not significant at other time points, a potential role of MPO for the observed changes in NO metabolism was supported by a reverse correlation of MPO sputum levels and FeNO. However, our observations are based on relatively low numbers and do not exclude the possibility that other mechanisms such as changes in airway pH, inflammation and increased NO synthase expression or function may contribute to the long-term effect of ivacaftor therapy on airway NO. The decreasing number of CF adults during follow up may also introduce a bias towards positive changes in FeNO over time, as the effect of ivacaftor on airway NO may be more pronounced in children compared to adults with CF (1).

In contrast to ivacaftor, lumacaftor-ivacaftor therapy did not result in a consistent change in FeNO. This may be related to differences in efficacy, as lumacaftor-ivacaftor it is known to have less significant effects on respiratory outcomes in patients homozygous for F508del, compared to ivacaftor mono-therapy in patients with gating mutations (10). The lack of effect on FeNO in children within the first year of treatment was consistent with a recent observation in 4 adults with CF (7). However, FeNO was significantly increased from baseline in a small number of our pediatric patients at two years follow-up (n=5). Whether longer term treatment with lumacaftor-ivacaftor results in a sustained effect on FeNO will need to be shown in future studies.

Conclusions

Ivacaftor results in a sustained increase in FeNO in children and adults with CF. The increase in FeNO with ivacaftor may be related to changes in airway NO-metabolism by MPO. Lumacaftor-ivacaftor did not have an immediate effect on FeNO in treated children, supporting findings in adults with CF.

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FIGURE LEGEND

Changes in the fraction of exhaled nitric oxide (FeNO) in patients with cystic fibrosis treated with ivacaftor or lumacaftor-ivacaftor. Each data point represent an individual measurement. Also shown are median and interquartile range (IQR) changes from before treatment baseline. Median FeNO was increased from baseline at all time points in ivacaftor treated patients. A significant increase in FeNO in the lumacaftor-ivacaftor treated patients was seen only at the 2 years follow-up.

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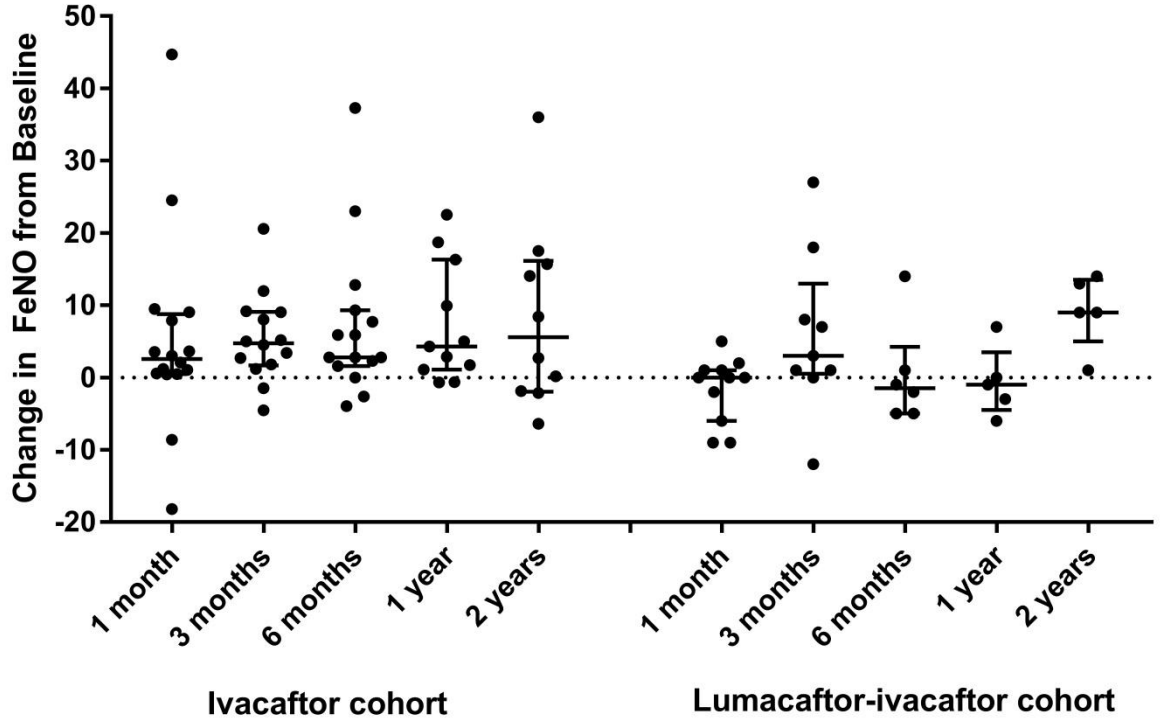
Figure


Change in FeNO from Baseline
50
40
30
20
10
0
-10
-20
1 month
3 months
6 months
1 year
2 years
1 month
3 months
6 months
1 year
2 years
Ivacaftor cohort
Lumacaftor-ivacaftor cohort