Variable cellular ivacaftor concentrations in people with cystic fibrosis on modulator therapy

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The development of CFTR modulators has transformed the care of patients with cystic fibrosis (CF). Although the clinical efficacy of modulators depends on their concentrations in target tissues, the pharmacokinetic properties of these drugs in epithelia are not utilized to guide patient care. We developed assays to quantitate ivacaftor in cells and plasma from patients on modulator therapy, and our analyses revealed that cellular ivacaftor concentrations differ from plasma concentrations measured concurrently, with evidence of in vivo accumulation of ivacaftor in the cells of patients. While the nature of this study is exploratory and limited by a small number of patients, these findings suggest that techniques to measure modulator concentrations in vivo will be essential to interpreting their clinical impact, particularly given the evidence that ivacaftor concentrations influence the activity and stability of restored CFTR protein.

1. Introduction

Mutations in the Cystic Fibrosis (CF) Transmembrane conductance Regulator gene (CFTR) lead to dysfunction of the CFTR ion channel [1]. To ameliorate this dysfunction, new drugs have been introduced: ivacaftor, a CFTR potentiator, used for surface localized, but dysfunctional CFTR channels [2]; lumacaftor, a CFTR corrector to partially correct the mutant F508del CFTR protein [3]; tezacaftor, an alternate corrector with a similar mechanism of action [4,5]; and most recently, elexacaftor, a next generation corrector used in combination with tezacaftor [6,7]. Ivacaftor is included in all clinically approved therapies alone and in combination with corrector compounds. For drugs that act within cells, tissue concentrations are superior to plasma concentrations to model pharmacodynamics [8–10]. As a hydrophobic molecule, ivacaftor has been shown to accumulate in the inner leaflet of plasma membranes [11] and in cultured airway epithelia [12]. Ivacaftor concentrations in epithelial tissues in vivo are a matter of debate, with some arguing that the concentrations used in most in vitro studies far exceed the in vivo concentrations [13,14]. These concentrations may be influenced by variability in plasma concentrations [15,16]. Understanding the concentrations of ivacaftor in target tissues is critical because high concentrations of ivacaftor are detrimental to CFTR rescue [12–18]. These findings suggest that determination of cellular and plasma ivacaftor concentrations would allow optimization of dosing and additional mechanistic studies. To address this issue, we developed a method to quantitate ivacaftor in plasma and epithelial cells obtained from patients on modulator therapy. We present a pilot study with a limited number of patients showing the feasibility of measuring ivacaftor in samples of cells directly from patients.

2. Methods

2.1. Patient samples

Written informed consent was obtained from nineteen subjects recruited under protocols approved by the institution’s Institutional Review Board (F151030001, University of Alabama at Birmingham). Plasma was collected from patients taking ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor, or on no modulator ther-

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apy. The timing of sample collection after ingestion of drug was not controlled. A subset of patients donated nasal epithelial cells via brush biopsy (sterile 5 mm cytobrush, Medical Packaging Corp., Panorama, CA). Cells were lysed with NP-40 lysis buffer. Samples were immediately processed with minimal handling and frozen at −80°C until analysis.

2.2. LC-MS/MS analysis

Reference standards were purchased from commercial sources providing certificates of analysis for all compounds compliant with FDA regulations: ivacaftor, tezacaftor, and lumacaftor (SelleckChem, USA); ivacaftor carboxylate (M6) and hydroxymethyl ivacaftor (M1) (Clearsynth Canada Inc). M1 and M6-ivacaftor were selected because manufacturer testing showed that these are disproportionately metabolites in humans and underwent further testing for regulatory approval [19]. Plasma assays were performed as previously described [20]. Plasma and whole-cell lysate were extracted as previously described [12–20]. Peak area ratio (PAR) was calculated as analyte relative to a stable internal standard and compared to a standard curve of seven spiked concentrations of compound in matrix (plasma or whole cell lysate). The resulting standard curve data was fit to a weighted linear regression of 1/x². Quality control (QC) samples across the concentration range were compared with the standard curve; ±15% difference between the QC and standard curve was considered acceptable. Dynamic range of the plasma assay is 1–1000 ng/mL whereas that of the lysate assay is 0.5–2000 ng/mL. The average cell volume (1.6 pl) of nasal epithelial cells was determined by measurement of cells. Lysate concentrations were normalized to total cell volume within the sample, calculated from the number of cells multiplied by average volume per cell. Inter-day assay replicate bias and imprecision were less than 8.36% for all three compounds; intra-day assay replicate bias and imprecision were less than 10.5% for the entire compound set. These values met the recommendations for bioanalytical methods by the FDA. We performed inter-day back calibration studies over a calibration range of 0.5–2000 ng/mL (R values >0.999 in all runs for all compounds).

3. Results

3.1. Cellular quantitation of ivacaftor

Cellular ivacaftor concentrations were measured in nasal biopsy samples from subjects on CFTR modulator therapy. Lysate concentrations were measured and normalized to estimated total cell volume in the sample (Table 1). Ivacaftor was detected in all subjects (range of 51.5–98.8 × 10⁶ ng/mL cell vol). M1-ivacaftor ( pharmacologically active) was detected in five subjects (range of 42.5–1.65 × 10⁵ ng/mL cell vol). M6-ivacaftor carboxylate (inactive metabolite) was not detected in any cellular samples, which may suggest an inability of these cells to produce the compound.

3.2. Comparison of plasma and cellular concentrations

Plasma concentrations of modulators were determined to assess their influence on the cellular concentrations of ivacaftor (Fig. 1, Table 2). The plasma concentrations for ivacaftor in the patients on monotherapy were 500–2780 ng/mL; on ivacaftor/lumacaftor 29.6–256 ng/mL; and ivacaftor/tezacaftor 180 and 635 ng/mL. The variation seen in these patients may be attributed to time after ingestion, and is within range of variation reported during regulatory review [19]. In patients taking ivacaftor/lumacaftor, measured values for lumacaftor were 1910–4950 ng/mL. In patients taking ivacaftor/tezacaftor, tezacaftor concentrations were 774 and 2070 ng/mL. No modulator compounds were detected in plasma from seven subjects not on CFTR modulators as controls. In six of seven subjects who also contributed nasal biopsy, the plasma concentration was lower than the cellular concentration (Table 2). A positive correlation was observed between plasma and cellular concentrations (Fig 1C, Spearman r = 0.78, p = 0.048). No metabolites of ivacaftor were detected in the cellular sample of the patients with highest cellular concentration of ivacaftor, which suggests decreased metabolism and clearance in the cells of the patients.

4. Discussion

This study shows substantial differences between the plasma and cellular ivacaftor concentrations, and suggest different patterns of metabolism in the epithelium compared to plasma. Although we observed a correlation between plasma and cellular concentrations, this relationship was not constant, with evidence of disproportionately elevated cellular concentrations in patients with higher plasma concentrations. The cellular concentrations observed are much higher than anticipated, but suggest in vivo accumulation of ivacaftor similar to prior in vitro reports [12,21] and to cellular accumulation of fluoroquinolones, which are similar in structure to ivacaftor [22]. These differences could result in a level of CFTR restoration in target tissues distinct from what might be expected from plasma concentrations. There is a need to measure cellular concentrations of ivacaftor and other modulators in vivo to understand patient exposures and place recent in vitro studies in context of patient response.

This study is limited by a small sample size and variable collection times after drug dosing, which likely affects our results. We suspect that cellular concentrations are likely more stable than plasma due to decreased metabolism. The cell volume used is estimated from measurements of cells in suspension, and is consistent with other reports of bronchial or nasal epithelial cell size [23,24].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell number</th>
<th>Ivacaftor</th>
<th>M1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw (ng/mL)</td>
<td>VN (ng/mL)</td>
<td>Raw (ng/mL)</td>
</tr>
<tr>
<td>A F508del/F508del</td>
<td>ivacaftor/lumacaftor</td>
<td>1 × 10⁶</td>
<td>2.38</td>
</tr>
<tr>
<td>B F508del/F508del</td>
<td>ivacaftor/lumacaftor</td>
<td>1 × 10⁶</td>
<td>0.68</td>
</tr>
<tr>
<td>F F508del/F508del</td>
<td>ivacaftor/tezacaftor</td>
<td>1 × 10⁶</td>
<td>3.8</td>
</tr>
<tr>
<td>G F508del/F508del</td>
<td>ivacaftor/lumacaftor</td>
<td>2.5 × 10⁶</td>
<td>148</td>
</tr>
<tr>
<td>H F508del/G551D</td>
<td>ivacaftor</td>
<td>1 × 10⁶</td>
<td>16.3</td>
</tr>
<tr>
<td>I F508del/G551D</td>
<td>ivacaftor</td>
<td>2.5 × 10⁶</td>
<td>327</td>
</tr>
<tr>
<td>J F508del/G551D</td>
<td>ivacaftor</td>
<td>2.5 × 10⁶</td>
<td>84</td>
</tr>
</tbody>
</table>

Lysate concentrations are measured by immediate lysis of counted cells after collection of nasal epithelial brush biopsy (raw). A known number of cells are lysed in lysis buffer for each subject (cell number). The raw concentrations are normalized to the estimated total volume of the cells in the sample. ND = Not Detected. VN = Volume Normalized.

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Fig. 1. Concentration of CFTR modulators. A. Cellular concentrations (open circles) of ivacaftor were normalized to average cell volume of human nasal epithelial cells (Table 1). For patients taking ivacaftor, with ingestion around the expected time of peak plasma concentration, there was wide variation in concentration. Ivacaftor was detected in all patients taking combination therapy (Table 1). B. Ivacaftor demonstrates variable concentrations consistent with expectations for the different drugs, and also variable concentrations among patients on the same therapy. Lumacaftor (black squares) is only detected in the patients taking ivacaftor/lumacaftor and tezacaftor (black diamonds) in those taking ivacaftor/tezacaftor. C. Non-parametric Spearman correlation analysis between cellular and plasma concentrations of ivacaftor. All values reflect ivacaftor concentrations, and the notation reflects the drug the patients are taking. Iva = Ivacaftor; Lum = Lumacaftor; Tez = Tezacaftor. Values plotted on log10 scale.

Table 2
Ivacaftor and metabolite concentrations in plasma of patients taking CFTR modulators.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>Compound</th>
<th>Time after last dose (hrs)</th>
<th>Ivacaftor (ng/mL)</th>
<th>P/C ratio</th>
<th>M1 (ng/mL)</th>
<th>M6 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F508del/F508del</td>
<td>Ivacaftor/lumacaftor</td>
<td>125</td>
<td>34.3</td>
<td>0.19</td>
<td>72.6</td>
<td>139</td>
</tr>
<tr>
<td>B</td>
<td>F508del/F508del</td>
<td>Ivacaftor/lumacaftor</td>
<td>14</td>
<td>29.6</td>
<td>0.57</td>
<td>129</td>
<td>409</td>
</tr>
<tr>
<td>C</td>
<td>F508del/F508del</td>
<td>Ivacaftor/lumacaftor</td>
<td>1</td>
<td>256</td>
<td>–</td>
<td>418</td>
<td>817</td>
</tr>
<tr>
<td>D</td>
<td>F508del/F508del</td>
<td>Ivacaftor/lumacaftor</td>
<td>8</td>
<td>114</td>
<td>–</td>
<td>464</td>
<td>2060</td>
</tr>
<tr>
<td>F</td>
<td>F508del/F508del</td>
<td>Ivacaftor/tezacaftor</td>
<td>14</td>
<td>180</td>
<td>0.63</td>
<td>350</td>
<td>225</td>
</tr>
<tr>
<td>G</td>
<td>F508del/F508del</td>
<td>Ivacaftor/tezacaftor</td>
<td>7</td>
<td>635</td>
<td>0.01</td>
<td>1660</td>
<td>1620</td>
</tr>
<tr>
<td>H</td>
<td>F508del/G551D</td>
<td>Ivacaftor</td>
<td>2.5</td>
<td>2780</td>
<td>2.26</td>
<td>3610</td>
<td>1520</td>
</tr>
<tr>
<td>I</td>
<td>F508del/G551D</td>
<td>Ivacaftor</td>
<td>4.25</td>
<td>804</td>
<td>0.01</td>
<td>3260</td>
<td>3780</td>
</tr>
<tr>
<td>J</td>
<td>F508del/G551D</td>
<td>Ivacaftor</td>
<td>6.5</td>
<td>500</td>
<td>0.02</td>
<td>783</td>
<td>201</td>
</tr>
<tr>
<td>Manufacturer's $C_{\text{max}}$*</td>
<td>Ivacaftor/lumacaftor</td>
<td>602</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Manufacturer's $C_{\text{max}}$*</td>
<td>Ivacaftor/tezacaftor</td>
<td>1170</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Manufacturer's $C_{\text{max}}$*</td>
<td>Ivacaftor</td>
<td>768</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Subjects without nasal biopsy are included to assess variation of concentrations and to compare to the manufacturer’s data. Not all subjects contributed a nasal biopsy (>). M1 = hydroxyethyl ivacaftor. M6 = ivacaftor carboxylate. *Manufacturer data from ivacaftor’s package insert provided for reference. $C_{\text{max}}$ = mean maximum concentration. For samples above the dynamic range (1–1000 ng/mL), they are diluted accordingly; dilutional integrity was confirmed ($\pm$3DEV). P/C is the plasma to cellular concentration ratio.

Epithelial cell volume varies significantly between and within individuals, which could potentially influence our conclusions. The small sample size and variation in therapies also precluded us from assessing whether differences in plasma or cellular concentrations were associated with differences in clinical outcomes following initiation of modulator therapy. Larger studies of patients with multiple timed samples at steady state are needed to fully assess any impact of cellular concentration on clinical outcomes. Nonetheless, these findings strongly suggest that such studies are needed to determine the impact of CFTR modulator concentrations in vivo, especially as larger populations of patients with CF take triple combination therapy including ivacaftor.

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Declaration of Competing Interest

S.M.R. serves as investigator and consultant to Vertex Pharmaceuticals, the manufacturer of ivacaftor, ivacaftor/lumacaftor, and ivacaftor/tezacaftor on CF clinical trials. J.S.G. and E.P.A. participated in clinical studies independent of Vertex Pharmaceuticals to evaluate the CFTR modulators described here. All other authors declare no conflicts of interest.

CRediT authorship contribution statement

Jennifer S. Guimbellot: Conceptualization, Data curation, Formal analysis, Project administration, Writing - original draft, Writing - review & editing. Kevin J. Ryan: Conceptualization, Data cu-
ration, Formal analysis, Writing - original draft, Writing - review & editing. **Justin D. Anderson**: Data curation, Project administration. **Zhongyu Liu**: Data curation, Project administration. **Latona Kersh**: Data curation, Project administration. **Charles R. Esther**: Writing - review & editing. **Steven M. Rowe**: Conceptualization, Writing - original draft, Writing - review & editing. **Edward P. Acosta**: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

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